201-15025

Karen Hoffman
01/05/04 09:33 AM

To: NCIC HPV@EPA, Michael Ofner/DC/USEPA/US@EPA
cc:
Subject: HPV Ref. No. 110 057 3 - Morflex, Inc.

----- Forwarded by Karen Hoffman/DC/USEPA/US on 01/05/04 09:32 AM -----

"Kennedy, Sam" <SKennedy@morflex.com >
12/29/03 04:10 PM

To: Rtk Chem@EPA
cc:
Subject: HPV Ref. No. 110 057 3 - Morflex, Inc.

Reference No. 110 057 3
Chemical: Acetyltributyl Citrate
CAS#: 77-90-7

Attached are the robust summaries and the reference appendix for the subject chemical. This data is being submitted to the US EPA per our commitment to sponsor this chemical in the High Production Volume Challenge Program.

If there are any questions relating to this submission, please contact me at your earliest convenience.

Sincerely,

Sam Kennedy
Morflex, Inc.
336-834-4933
skennedy@morflex.com

ATBC HPV Assessment and Test Plan 121603 fina ATBC HPV Appendix (Robust Summaries) 121603 fin

201-15025

U.S. EPA HIGH PRODUCTION VOLUME (HPV)
CHEMICALS CHALLENGE PROGRAM

Assessment of Data Availability and Test Plan for Acetyl Tributyl Citrate (ATBC) (CAS RN 77-90-7)

Prepared for:

Morflex, Inc.

Prepared by:

Toxicology/Regulatory Services, Inc.

December 16, 2003

Assessment of Data Availability and Test Plan for Acetyl Tributyl Citrate (ATBC) (CAS RN 77-90-7)

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CHEMICAL IDENTITY AND USE INFORMATION

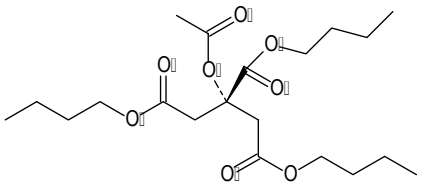
CAS RN

77-90-7

Chemical Name

Acetyl Tributyl Citrate

Structure, Molecular Formula, Molecular Weight



Molecular Formula: $C_{20}H_{34}O_8$
Molecular Weight: 402.5

OTHER CHEMICAL IDENTITY INFORMATION

ATBC
Tributyl O-acetylcitrate
1,2,3-Propanetricarboxylic acid, 2-(acetyloxy)-, tributyl ester
Citric acid, tributyl ester, acetate
Citroflex® A-4

USE PATTERN

Acetyl tributyl citrate (ATBC) is used as a plasticizer with aqueous- and solvent-based polymers, including acrylic, methacrylic, ethyl cellulose, hydroxypropyl methyl cellulose, nitrocellulose, vinyl acetate, vinyl chloride, vinyl pyrrolidone, vinylidene chloride, and urethane polymer systems. ATBC is used in the following applications:

- Medical plastics: Aqueous pharmaceutical coatings; extra-corporeal tubing.
- Food contact products: Food wraps and films; beverage tubing; crown liners; food containers; tinplate lubricant; aluminum foil coatings.
- Cellulosics: Nitrocellulose-based explosives/propellants.
- Other industrial uses: Children's toys; animal ear tags; ink formulations; adhesives; pesticide inerts.

AVAILABLE DATA TO FULFILL HPV SCREENING INFORMATION DATA SET (SIDS) ENDPOINTS

Table 1: Test Plan

ACETYL TRIBUTYL CITRATE CAS RN: 77-90-7		Information	Guideline Study	GLP	Other Studies Available	Estimation Method	Acceptable	Testing Required
SIDS Endpoint	**STUDY**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL/CHEMICAL PROPERTIES DATA								
2.1	Melting Point	Y	N	N	Y	Y	Y	N
2.2	Boiling Point	Y	N	N	Y	Y	Y	N
2.4	Vapor Pressure	Y	N	N	Y	Y	Y	N
2.5	Partition Coefficient	Y	N	N	Y	Y	Y	N
2.6	Water Solubility	Y	N	N	Y	Y	Y	N
ENVIRONMENTAL FATE DATA								
3.1.1	Photodegradation	Y	N	N	N	Y	Y	N
3.1.2	Stability in Water	Y	N	N	N	Y	Y	N
3.3.2	Transport and Distribution	Y	N	N	N	Y	Y	N
3.5	Biodegradation	Y	Y	N	Y	Y	Y	N
ECOTOXICITY DATA								
4.1	Acute/Prolonged Toxicity to Fish	Y	Y	Y	Y	Y	Y	N
4.2	Acute Toxicity to Aquatic Invertebrates	Y	Y	Y	N	Y	Y	N
4.3	Toxicity to Aquatic Plants, e.g. Algae	Y	N	N	N	Y	Y	N
HUMAN HEALTH-RELATED DATA								
5.1.1	Acute Toxicity	Y	N	N	Y	N	Y	N
5.4	Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
5.5	Genotoxicity *In Vitro* (Bacterial Test)	Y	Y	Y	Y	N	Y	N
	Genotoxicity *In Vitro or In Vivo* (Chromosome Aberration Tests)	Y	Y	Y	Y	N	Y	N
	Genotoxicity *In Vitro* (Mammalian Cells)	Y	Y	Y	Y	N	Y	N
5.8	Reproductive Toxicity	Y	Y	Y	Y	N	Y	N
5.9	Development Toxicity / Teratogenicity	Y	N	N	Y	N	Y	N

Note: Additional studies include an *in vivo*/*in vitro* unscheduled DNA synthesis study (Appendix, *5.5 Genetic Toxicity In Vitro*), a rat adsorption, metabolism and excretion study and two *in vitro* metabolism studies (Appendix, *5.10 Additional Studies*).

Approach to Evaluate the Database for Acetyl Tributyl Citrate (ATBC)

The following approach was used to obtain and analyze data relevant to the assessment of ATBC.

1. The chemical name and CAS RN of ATBC were provided by Morflex, Inc.
2. Published and unpublished reports were obtained from Morflex, Inc. and other sources; they were organized and reviewed to identify studies that could fulfill SIDS endpoints.
3. Pertinent publicly available databases[1] were searched and all relevant reports were obtained to establish the full extent and nature of the published literature for ATBC.
4. A references database was developed and maintained in order to track reports through the review, assessment and summarization process.
5. Each of the reports obtained was reviewed to determine adequacy according to EPA criteria and reliability according to Klimisch *et al.* (1997).
6. Robust Summaries were prepared for each report with a Klimisch score of 1 or 2, in accordance with the guidelines proposed by the EPA (U.S. EPA, 1999a) for each study type.
7. Physical/chemical properties and environmental fate and ecotoxicity data were estimated by using appropriate Quantitative Structure Activity Relationships (QSARs) (U.S. EPA, 1999b).
8. Fugacity modeling (Level III) was performed to estimate transport and distribution of ATBC into environmental compartments (U.S. EPA, 2000a; Mackay *et al*., 1996a,b).

Use of Structure Activity Relationships for Acetyl Tributyl Citrate (ATBC)

Approaches recommended in the EPA document on the use of structure activity relationships (SAR) in the HPV Chemicals Challenge Program were employed in the assessment of ATBC (U.S. EPA, 1999b). Several SAR-based models, as well as Mackay-type fugacity-based modeling, were employed to support the review and assessment of ATBC. The SAR models for physical properties were used to estimate boiling points, melting points, aqueous solubilities, octanol/water partition coefficients and vapor pressures. Other SAR models were used to estimate hydroxyl radical-mediated atmospheric photo-oxidation and biodegradation potential. SAR models also were used to obtain estimates of acute toxicity to aquatic organisms.

Physical/Chemical Properties QSAR Estimates and Correlation to Reliable Data

Robust Summaries for available reliable studies and QSAR estimates for physical/chemical properties of ATBC are presented in the Appendix.

[1] Databases include ChemIDplus, HSDB (Hazardous Substances Data Bank), IRIS (Integrated Risk Information System), CCRIS (Chemical Carcinogenesis Research Information System), GENE-TOX, EMIC (Environmental Mutagen Information Center), DART/ETIC (Developmental and Reproductive Toxicology and Environmental Teratology Information Center), MEDLINE, TOXLINE, RTECS (Registry of Toxic Effects of Chemical Substances), TSCATS (Toxic Substances Control Act Test Submissions), and IUCLID (International Uniform Chemical Information Database), 1996.

Where possible, the physical/chemical property estimation program EPIWIN version 3.10 was used to derive estimates. QSAR estimates are based on structure and, therefore, can be made only for substances for which a structure can be defined. Since ATBC has a defined structure, a complete set of model data was generated. In general, EPIWIN estimates must be interpreted with a great deal of professional judgment; however, the model estimates for the physical/chemical properties of ATBC are, in most cases, comparable to the available reliable measured data. The available data for physical/chemical properties are summarized below.

Measured data for melting and boiling points were –59°C and 326°C at 760 mm Hg, respectively. The EPIWIN MPBPWIN model-predicted values (U.S. EPA, 2000b) were comparable to the measured data with predicted melting and boiling points of –94.35°C and 410.75°C, respectively. The EPIWIN model also provided an experimental database match for melting point of –80°C, which corroborates the measured and predicted values for melting point.

Vapor pressure was measured as 0.052 mm Hg at 20°C, whereas the EPIWIN MPBPWIN (U.S. EPA, 2000b) model-predicted value was 0.000485 mm Hg at 25°C.

The octanol/water partition coefficient (log K_{ow}) was determined using HPLC to be 4.92 at 22°C and the EPIWIN KOWWIN model-predicted value (U.S. EPA, 2000c) was 4.29 at 25°C.

Measured data and the EPIWIN WSKOWWIN model prediction (U.S. EPA, 2000d) for water solubility were <100 mg/l and 2.045 mg/l at 25°C, respectively. The EPIWIN WSKOWWIN model output also provided an experimental database match of 5 mg/l, which corroborates the predicted value for water solubility.

Environmental Fate and Ecotoxicity QSAR Estimates and Correlation to Reliable Data

Robust Summaries for the reliable studies and QSAR estimates for the environmental fate and effects of ATBC are presented in the Appendix.

The model for atmospheric photodegradation was run according to EPA guidelines. Modeling with EPIWIN AOPWIN (U.S. EPA, 2000e) indicated that ATBC would be expected to degrade rapidly ($t_{½}$ = 0.740 days) upon exposure to ambient light.

The water stability of ATBC was determined using the EPIWIN HYDROWIN model (U.S. EPA, 2000f) and found to be pH dependent. At 25°C and pH 7, the K_b $t_{½}$ = 3.816 days and, at 25°C and pH 8, the K_b $t_{½}$ = 139.394 days. This allows one to conclude that ATBC is moderately stable in water under environmental conditions.

Modeling for environmental transport and distribution (EPIWIN Level III fugacity model, Mackay-type; U.S. EPA, 2000a) predicted distribution to air (mass amount 99%; $t_{½}$ = 17.8 hours) and no appreciable distribution to water, soil or sediment following entry of ATBC into the environment *via* air emissions (1000 kg/hr).

Regarding biodegradation, measured data exist from several studies conducted in various media. The test types and results are summarized in the following table.

Table 2: Summary of Biodegradation Studies

Test Type and Contact Time	Inoculum	Degradation	Results
Standard BOD test 21 days	Unacclimated sewage treatment organisms	Slow rate of degradation under stringent test conditions	100 × (BOD/Chemical Oxygen Demand): Day 5 = 14% Day 21 = 26%
Sewage column degradation 5 hours	Acclimated sewage column sludge	Rapidly biodegradable	> 90% biodegradation in 5 hours
Aerobic biodegradation in soil using a static biometer system 42 days	Soil organisms	Readily biodegradable	Mineralization reached 166.8, 124.4, 97.2, 97.4 and 72.9% as $ThCO_2$ by day 42 in test vessels containing 0.8, 1.6, 3.2, 6.0 and 12.0 mg C/g soil, respectively
Aerobic biodegradation in soil 52 days	Commercial compost seed	Rapidly biodegradable	Mineralization by day 52 reached 128, 125, 90 and 83% $ThCO_2$ for 40, 80, 160 and 300 mg C-treatments, respectively
Ultimate biodegradation in actively aerated compost 45 days	Commercial BOD seed inoculum	Ultimately biodegradable	Mineralization reached 37% $ThCO_2$ in 45 days
Respirometry test in static compost biometer system 28 - 44 days	Compost microorganisms	Readily biodegradable	At 10.8 mg-C/g dry soil, conversion to CO_2 exceeded 60% $ThCO_2$ in three weeks; at 1.9 mg-C/g dry soil, conversion to CO_2 exceeded 60% $ThCO_2$ within four days following the lag period
EPIWIN BIOWIN model (U.S. EPA, 2000g) Not applicable	Not applicable	Fast; readily degradable	$t_{½}$ (water) = 8.67 days $t_{½}$ (soil) = 8.67 days $t_{½}$ (sediment) = 34.67 days

Reliable measured data were available for fish and aquatic invertebrates, and EPIWIN ECOSAR estimates (U.S. EPA, 2000h) for acute fish, daphnid and algal toxicity were modeled. Reliable measured data for acute toxicity to fish were available for mummichogs (*Fundalus heteroclitus*) and bluegill sunfish (*Lepomis macrochirus*) with 96-hour LC_{50} values ranging from 38 – 60 mg/l (nominal concentrations). The 7-day EC_{50} values based on survival and growth of the fathead minnow (*Pimephales promelas*) were 1.9 and 1.4 mg/l, (mean measured test concentrations) respectively. The EPIWIN ECOSAR 96-hour LC_{50} for acute toxicity to the fathead minnow (*Pimephales promelas*) was predicted as 1.669 mg/l. Reliable measured data for acute toxicity to aquatic invertebrates was available for the water flea (*Ceriodaphnia dubia*) with a 48-hour EC_{50} value of 7.82 mg/l (mean measured test concentration). The EPIWIN ECOSAR 48-hour LC_{50} for acute toxicity to the daphnid (*Daphnia magna*) was predicted as 0.704 mg/l. The EPIWIN ECOSAR 96-hour EC_{50} for acute toxicity to green algae (*Selenastrum capricornutum)* was predicted as 0.148 mg/l. Based on the physical/chemical properties of ATBC and the reliable measured 48-hour and 7-day EC_{50} values (as mean

measured test concentration) for the water flea (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*), respectively, the predicted EPIWIN ECOSAR values appear to be conservative estimates of the toxicity of ATBC to aquatic species.

Human Health-Related Reliable Data

Robust Summaries for the reliable human health-related studies with ATBC are presented in the Appendix.

Acute oral toxicity data are available for ATBC in rats and cats with LD_{50} values >30 and >50 ml/kg, respectively. Although acute dermal toxicity data are not available, ATBC is predicted to present a very low potential for toxicity *via* the dermal route of exposure because of its extremely high oral LD_{50} and because it is unlikely to be absorbed efficiently through the skin. Acute inhalation toxicity data are not available, but ATBC is predicted to present a very low potential for toxicity *via* the inhalation route exposure because of its low vapor pressure and extremely high oral LD_{50}.

ATBC was studied for repeated dose toxicity in rats, cats and mice in numerous studies ranging in duration from 14 days to 2 years. High doses of ATBC were administered *via* the dietary, oral gavage or intraperitoneal injection routes of exposure. ATBC was shown to possess a low level of subchronic toxicity in rats and cats *via* the oral route of exposure. At very high doses, the few minor changes seen were considered to be a reflection of metabolic adaptation or incidental rather than toxic effects. In two-year oral toxicity studies in rats, ATBC was shown to possess a low level of chronic toxicity potential. No treatment-related toxic effects were reported after two years of daily exposure to high levels of ATBC. Organ specific toxicity was not reported in any of these studies and in all cases the NOAELs are greater than or equal to 100 mg/kg bw/day based on general toxicity associated with high dose exposure regimens.

The numerous testing results for repeated dose toxicity studies summarized in the following table support the conclusion that ATBC has a low potential to cause systemic toxicity.

Table 3: Summary of Repeated Dose Toxicity Studies

Species; Sex	Route	No. of Treatment Groups / No. of Animals per Group	Duration	NOAEL (mg/kg bw/day)
Rat; M & F	Oral (feed)	2 / 4	6 weeks	5%
Rat; M & F	Oral (feed)	2 / 4	8 weeks	10%
Cat; NS	Oral (gavage)	1 / 2	2 months	5250 [a]
Mouse; NS	I.P. injection	1 / 5	14 days	<900
Rat; M & F	Oral (feed)	3 / 10	14 days	<1000
Rat; M & F	Oral (feed)	3 / 40	90 days	300
Rat; NS	Oral (feed)	3 / 20	2 years	100
Rat; M & F	Oral (feed)	3 / 50	*In utero* exposure phase + 90 days	M = 100 F = 300

M = Male; F = Female
NS = Not stated
[a] Assumes a specific gravity of 1.05.

The available battery of six negative *in vitro* genotoxicity assays is adequate to conclude that ATBC does not pose a genotoxicity concern for humans. In addition, ATBC was shown to

be negative in an *in vivo*/*in vitro* UDS study, which further indicates an absence of *in vivo* genotoxic potential for ATBC.

The *in vitro* and *in vivo* testing results summarized in the following table support the conclusion that ATBC is neither mutagenic nor genotoxic.

Table 4: *In vitro* and *In vivo* Mutagenicity/Genotoxicity Studies for ATBC

Test System	Test Object	Concentration of Substance	Results
Ames assay (preincubation method)	*S. typhimurium* TA98, TA100, TA1535, TA1537	50 to 5000 µg/plate	Negative[a,b]
Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	333 to 10,000 µg/plate	Negative[a,b]
Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	9 to 495 µg/plate	Negative[b]
In vitro chromosomal aberration assay	Rat lymphocyte cells	4 to 400 µg/ml	Negative[a,b]
In vivo/in vitro unscheduled DNA synthesis	Male rat primary cultures of hepatocytes	Single oral doses of 800 or 2000 mg/kg	Negative
Forward mutation assay	L5178Y (TK+/TK-) mouse lymphoma cells	200 to 480 µg/ml[a] 10 to 230 µg/ml[b]	Negative[a,b]
Forward mutation assay	CHO/HGPRT	25 to 400 µg/ml	Negative[a,b]

[a] With metabolic activation.
[b] Without metabolic activation.

Evaluation of potential for reproductive effects is satisfied for ATBC by a two-generation reproduction study and a 13-week toxicity study with an *in utero e*xposure phase.

In a two-generation reproduction study, no treatment-related clinical observations were noted throughout the study in either F_0 or F_1 parental animals. Body weights of F_0 parents and F_1 females were largely unaffected by treatment with ATBC; however, body weights of the F_1 parental males in the 300 and 1000 mg/kg bw/day groups were consistently lower that controls and appeared to be related to treatment. Body weights of the F_0 females in the 1000 mg/kg bw/day group at the end of pregnancy (gestation days 21 or 22) were significantly lower than control values. Water consumption of the F_0 and F_1 parental animals fed ATBC at a level of 1000 mg/kg bw/day were consistently lower than concurrent controls throughout the study. Mating, gestation and fertility of the F_0 and F_1 generations were unaffected by treatment. There were no abnormalities seen at necropsy that were considered to be treatment-related. The body weights of the pups from the 300 and 1000 mg/kg bw/day dose groups were slightly lower than those of the controls, and slightly higher mortality also was observed in these groups. These effects were considered to be a consequence of the reduced water intakes in the dams at these dose levels rather than a direct effect of ATBC. No other treatment-related effects were observed in the parameters evaluated. Parental and offspring NOAELs both were 100 mg/kg bw/day based on slight body weight effects in the mid- and high-dose groups.

In a 13-week toxicity study with an *in utero e*xposure phase, sensitive reproductive and developmental endpoints were examined. Parental animals were evaluated for reproductive

endpoints (mating performance, fertility, gestation length and parturition, litter size, numbers of implantations, survival and growth), and F_1 offspring were evaluated for sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males, sperm assessments and estrous cyclicity), as well as physical appearance, ophthalmologic effects, neurobehavioral effects, growth, food consumption, survival, hematology, blood chemistry, urinalysis, peroxisome proliferation, organ weights, gross pathology and histopathology. Estrous cycles, mating performance, fertility, gestation length and parturition, were all unaffected by treatment. Litter size, survival and growth were similar in all groups and within expected historical control ranges. Although numbers of implantations and litter size at 1000 mg/kg bw/day were marginally lower than concurrent control group levels, they were within the laboratory's historical control ranges. Anogenital distance and sexual maturation in both sexes and retention of areolae in male offspring were unaffected by treatment. There were no adverse effects on sperm motility, counts or morphology. There were no findings at necropsy of parental animals or surplus offspring that were considered to be treatment-related. Parental and offspring NOAELs were 300 and 1000 mg/kg bw/day for reproductive and developmental endpoints, respectively.

ATBC was evaluated for developmental toxicity potential in 12-month studies conducted with rats and mice. Groups of rats and mice were provided feed which contained a milk solution of the test substance (ATBC) at doses of 50 and 250 mg/kg bw/day for 12 months. A third group served as a control. In the ninth month of the study, a cross-mating of the animals was performed, male gonads were evaluated and embryotoxic effects were examined. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (determined by examining the numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings, eye openings, appearance of body hair and teeth, behavior and body weight. In both species there were no effects of treatment noted at doses of 50 mg/kg bw/day. ATBC had no significant effects in rats or mice on male gonads, and the spermatogenesis index in animals of the 250 mg/kg bw/day group was similar to controls. Increases in body weight and length of the progeny and placental weight were observed in the 250 mg/kg bw/day dose group. There were no differences between groups in the fertility rate and number of animals born per pregnant female. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of the progeny also were unaffected by treatment. The developmental toxicity NOAEL in both studies was 250 mg/kg bw/day.

Also regarding developmental toxicity, developmental effects were not observed at dose levels as high as 1000 mg/kg bw/day in a two-generation reproduction study nor in a 13-week toxicity study with an *in utero e*xposure phase. ATBC is rapidly and extensively absorbed, and then rapidly metabolized and virtually completely excreted by the rat. The metabolites that have been positively identified in the urine of rats (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and two isomers of acetyl dibutyl citrate) have been demonstrated to undergo rapid clearance from the body and are not suspected to be developmental toxicants. Also, other ATBC metabolites, acetic acid, citric acid, butyric acid, tributyl citrate and butanol, do not pose a concern for developmental toxicity (see *5.8 Toxicity to Reproduction* and *5.10 Additional Studies*).

SUMMARY OF TEST PLAN

The test plan for physical/chemical properties is summarized in Table 1. Morflex, Inc. contends that the existing measured and modeled data for melting point, boiling point, vapor pressure, octanol/water partition coefficient, and water solubility are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program. No additional data development is suggested for ATBC.

The environmental fate and ecotoxicity test plan is summarized in Table 1. Morflex, Inc. contends that the existing measured and modeled data for photodegradation, stability in water, transport and distribution, biodegradation, acute toxicity to fish, acute toxicity to aquatic invertebrates, and toxicity to aquatic plants are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program. No additional data development is suggested for ATBC.

The human health-related test plan is summarized in Table 1. Morflex, Inc. contends that the existing measured data for acute toxicity, repeated dose toxicity, genotoxicity *in vitro* and *in vivo*, reproductive toxicity, and development toxicity are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program, especially when the ATBC rat adsorption, metabolism and excretion study and two *in vitro* metabolism studies also are considered. No additional data development is suggested for ATBC.

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Mackay, D., A. Di Guardo, S. Paterson, G. Kicsi and C.E. Cowan (1996a) Assessing the fate of new and existing chemicals: A five-stage process. *Environ. Toxicol. Chem*., **15(9)**, 1618-1626.

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U. S. EPA. (1999b) The Use of Structure-activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. http://www.epa.gov/chemrtk/sarfinl1.htm.

U.S. EPA. (2000a) EPI Suite™, Version 3.10; Mackay's Equilibrium Concentration Model (EQC) Fugacity Model, LEVEL3NT (v 1.01); PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000b) EPI Suite™, Version 3.10; MPBPWIN Program, Version 1.40; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000c) EPI Suite™, Version 3.10; KOWWIN Program, Version 1.66; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000d) EPI Suite™, Version 3.10; WSKOWWIN Program, Version 1.36; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000e) EPI Suite™, Version 3.10; AOPWIN Program, Version 1.90; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000f) EPI Suite™, Version 3.10; HYDROWIN Program, Version 1.67; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000g) EPI Suite™, Version 3.10; Biodegradation Probability Program (BIOWIN), Version 4.00; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000h) EPI Suite™, Version 3.10; ECOSAR Version 0.99g; PC-Computer software developed by ECOSAR Program, Risk Assessment Division (7403), Washington, D.C.

201-15025

Robust Summaries of Reliable Studies and QSAR Model Data
Acetyl Tributyl Citrate (ATBC)

Appendix

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Acetyl Tributyl Citrate

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2.1 MELTING POINT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated (99.0%, minimum specification)

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Remarks:	Determined by Morflex, Inc.

Results

Melting Point:	-59 °C
Decomposition:	Not applicable
Sublimation:	Not applicable
Remarks:	(Reported as a pour point.)

Conclusions The endpoint has been adequately characterized by Morflex, Inc.

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; company data.

References

Morflex, Inc. 1998. Citroflex® Citric Acid Esters, Technical Bulletin 101, Morflex, Inc., Greensboro, NC, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.1 MELTING POINT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) MPBPWIN Program (v 1.40) – Estimated value was obtained using the Joback Group Contribution Method and Gold and Ogle Method.
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Melting Point:	Estimated (weighted mean) = -94.35 °C Experimental database match = -80 °C (Sandmeyer and Kirwin, 1981)
Decomposition:	Not applicable
Sublimation:	Not applicable
Remarks:	

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; MPBPWIN Program, Version 1.40; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.2 BOILING POINT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated (99.0%, minimum specification)

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Remarks:	Determined by Morflex, Inc.

Results

Boiling Point:	326°C
Pressure:	760
Pressure Unit:	mm Hg
Decomposition:	None observed
Remarks:	

Conclusions The endpoint has been adequately characterized by Morflex, Inc.

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; company data.

References

Morflex, Inc. 1998. Citroflex® Citric Acid Esters, Technical Bulletin 101, Morflex, Inc., Greensboro, NC, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.2 BOILING POINT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) MPBPWIN Program (v 1.40) – Estimated value was obtained using the adapted Stein and Brown method.
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Boiling Point:	Estimated = 410.75°C Experimental database match = 172°C (at 1 mmHg) (Sandmeyer and Kirwin, 1981)
Pressure:	760
Pressure Unit:	mm Hg
Decomposition:	Not applicable
Remarks:	

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; MPBPWIN Program, Version 1.40; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.4 VAPOR PRESSURE

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated (99.0%, minimum specification)

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Remarks:	Determined by Morflex, Inc.

Results

Vapor Pressure:	5.2×10^{-2} mm Hg
Temperature:	20°C
Decomposition:	None observed
Remarks:	

Conclusions The endpoint has been adequately characterized by Morflex, Inc.

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; company data.

References

Morflex, Inc. 1998. Citroflex® Citric Acid Esters, Technical Bulletin 101, Morflex, Inc., Greensboro, NC, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.4 VAPOR PRESSURE

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) MPBPWIN Program (v 1.40) – Estimated value was obtained using the Modified Grain method.
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Vapor Pressure:	4.85×10^{-4} mm Hg
Temperature:	25°C
Decomposition:	Not applicable
Remarks:	

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; MPBPWIN Program, Version 1.40; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.5 PARTITION COEFFICIENT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated (99.0%, minimum specification)

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	2001
Remarks:	Determined by Morflex, Inc. with HPLC.

Results

Log K_{ow}:	4.92
Temperature:	22°C
Remarks:	

Conclusions The endpoint has been adequately characterized by Morflex, Inc.

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; company data.

References

Morflex, Inc. 1998. Citroflex® Citric Acid Esters, Technical Bulletin 101, Morflex, Inc., Greensboro, NC, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.5 PARTITION COEFFICIENT

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method:	EPIWIN (v 3.10), KOWWIN Program (v 1.66)
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Log K_{ow}:	4.29
Temperature:	25°C
Remarks:	

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; KOWWIN Program, Version 1.66; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.6 WATER SOLUBILITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated (99.0%, minimum specification)

Method

Method/Guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Remarks:	Determined by Morflex, Inc.

Results

Solubility:	< 100 mg/l
Temperature:	Not stated
pH value and concentration:	Not stated
pKa value at 25°C:	Not stated
Remarks	

Conclusions The endpoint has been adequately characterized by Morflex, Inc.

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; company data.

References

Morflex, Inc. 1998. Citroflex® Citric Acid Esters, Technical Bulletin 101, Morflex, Inc., Greensboro, NC, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

2.6 WATER SOLUBILITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10), WSKOWWIN Program (v 1.40)
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C, melting point of -59°C and molecular weight of 402.49.

Results

Solubility:	Estimated = 2.045 mg/L Experimental database match = 5 mg/L (Chem. Inspect. Test Institute, 1992)
Temperature:	25°C
pH value and concentration:	Not applicable
pKa value at 25°C:	Not applicable
Remarks	

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; WSKOWWIN Program, Version 1.36; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	

3.1.1 PHOTODEGRADATION

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/guideline followed:	EPIWIN (v 3.10), AOPWIN Program (v 1.90)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Concentration of substance:	Not applicable
Temperature °C:	Not Stated
Direct photolysis:	Not applicable
Indirect photolysis:	Not applicable
Breakdown products:	Not applicable
Remarks:	Overall OH Rate Constant (k_{phot}) = 14.4562 E-12 cm^3/molecule-sec $t_{1/2}$ = 0.740 days (12-hour day; 1.5 E6 OH/cm^3)

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data

References U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; AOPWIN Program, Version 1.90; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

3.1.2 STABILITY IN WATER

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/guideline followed:	EPIWIN (v 3.10), HYDROWIN Program (v 1.67)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Total K_b:	5.755 x 10^{-2} L/mol·sec
pH:	> 8
Temperature:	25°C
K_b Half-life at pH 8:	139.394 days
K_b Half-life at pH 7:	3.816 days
Remarks:	Fragment(s) on this compound were not available from the fragment library. Substitute(s) were used.

Conclusions The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; HYDROWIN Program, Version 1.67; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other

Last changed:	December 8, 2003
Order number for sorting:	
Remarks:	

3.3.2 TRANSPORTATION BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY MODEL)

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable
Remarks:	

Method

Method/Guideline followed:	EPIWIN (v 3.10) Level III Fugacity Model; adapted from Mackay's EQC Level III Fugacity Model
Media:	Water, air, soil and sediment (model run with 1000 kg/hr emissions to air)
GLP:	Not applicable
Year:	2003
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Remarks: Following are the results from the model:

```
Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : 1,2,3-Propanetricarboxylic acid, 2-(acetyloxy)-,
                tributyl ester
  Molecular Wt: 402.49
  Henry's LC  : 0.00551 atm-m3/mole (calc VP/Wsol)
  Vapor Press : 0.052 mm Hg  (user-entered)
  Log Kow     : 4.92  (user-entered)
  Soil Koc    : 3.41e+004  (calc by model)

            Mass Amount    Half-Life    Emissions
             (percent)        (hr)       (kg/hr)
   Air       99              17.8         1000
   Water     0.309           208          0
   Soil      0.555           208          0
   Sediment  0.0872          832          0

             Fugacity    Reaction    Advection   Reaction    Advection
              (atm)      (kg/hr)      (kg/hr)    (percent)   (percent)
   Air       1.24e-011    795         204         79.5         20.4
   Water     4.12e-012    0.212       0.0636      0.0212       0.00636
   Soil      1.06e-013    0.381       0           0.0381       0
   Sediment  7.49e-013    0.0149      0.000359    0.00149      3.59e-005

   Persistence Time: 20.6 hr
   Reaction Time:    25.9 hr
   Advection Time:   101 hr
   Percent Reacted:  79.6
   Percent Advected: 20.4
```

```
Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
   Air:      17.76
   Water:    208.1
   Soil:     208.1
   Sediment: 832.3
     Biowin estimate: 3.553  (days-weeks  )

Advection Times (hr):
   Air:      100
   Water:    1000
   Sediment: 5e+004
```

Conclusions:

Mass Amounts:
Air = 99 %
Water < 1%
Soil < 1 %
Sediment < 0.1%

Remarks: The endpoint has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability: 2D
Remarks: Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; Level III Fugacity Model; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks:

3.5 BIODEGRADATION

Test Substance

Identity:	Citroflex-A4 (O-acetyl-tributyl citrate) (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated
Remarks:	

Method

Method/Guideline followed:	Not stated
Test Type:	Standard BOD test; oxygen uptake
GLP:	Not stated
Year:	1974
Contact Time:	21 days
Inoculum:	Unacclimated sewage treatment organisms
Analytical Monitoring:	Yes; GC.
Remarks:	

Results

Degradation:	Test substance was characterized as having a slow rate of degradation under stringent test conditions.
Results:	100 x (BOD/Chemical Oxygen Demand): Day 5 = 14% Day 21 = 26%
Kinetic:	Not stated.
Breakdown Products:	Not stated.

Conclusions Biodegradability as a measure of BOD has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References Foulds, G. 1974. Potential Environmental Impact of Citroflexes. Unpublished report. Pfizer Ecosystems Laboratory, Groton, CT, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	[BOD test]

3.5 BIODEGRADATION

Test Substance

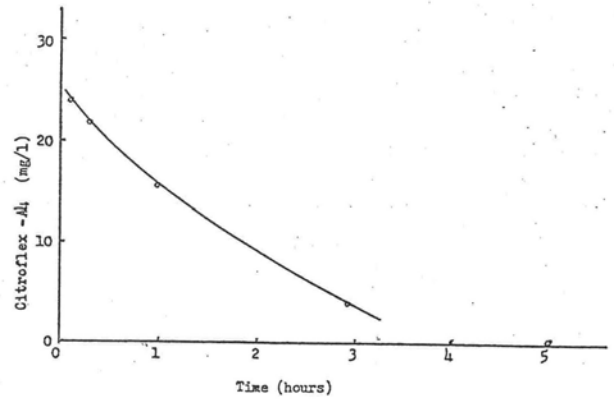
Identity:	Citroflex-A4 (O-acetyl-tributyl citrate) (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated
Remarks:	

Method

Method/Guideline followed:	Not stated
Test Type:	Sewage column degradation
GLP:	Not stated
Year:	1974
Contact Time:	5 hours
Inoculum:	Acclimated sewage column sludge
Analytical Monitoring:	Yes; GC
Remarks:	The sewage columns were acclimated to the test substance by exposure at low concentrations for 3 to 4 days prior to study initiation. Biodegradability of this test substance under aerobic conditions was monitored over five hours.

Results

Degradation:	This test substance was rapidly biodegraded in acclimated sewage columns.
Results:	>90% biodegradation in 5 hours.
Kinetic:	Provided graphically below.
Breakdown Products:	Not stated
Remarks:	The following degradation curve illustrates the disappearance of the test substance by the acclimated sewage column sludge over time (as presented by the author):



Conclusions

Potential biodegradability of the test substance as a factor of degradation in sewage treatment conditions has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability: 2C
Remarks: Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References

Foulds, G. 1974. Potential Environmental Impact of Citroflexes. Unpublished report. Pfizer Ecosystems Laboratory, Groton, CT, USA.

Other Available Reports

Other

Last Changed: December 3, 2003
Order Number for Sorting:
Remarks: [Sewage column degradation]

3.5 BIODEGRADATION

Test Substance

Identity:	Acetyltri-n-butyl Citrate; ATBC (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated (99.0%, minimum specification)
Remarks:	

Method

Method/Guideline followed:	EPA method OPPTS 835.3300; ASTM D 5988
Test Type:	Aerobic biodegradation in soil using a static biometer system.
GLP:	Not stated
Year:	2000
Contact Time:	42 days
Inoculum:	Soil organisms
Statistical Analyses:	Nonlinear regression techniques were applied to mineralization curves to fit a 'three parameter, single exponential rise to a maximum' model.
Remarks:	A standard laboratory soil mix was prepared, characterized, adjusted to 60% of its water holding capacity (WHC) and preincubated for 48 hours. The soil was amended with 67 to 1149 mg test substance per 50 g dry soil and placed in a 250-ml reactor vessels fitted with a side-arm chamber charged containing 20 ml of 0.5-M KOH. Reactors were similarly prepared for positive (cellulose) and negative controls. Reactors were prepared in triplicate for each treatment, stoppered and incubated in the dark at 30±1°C for 42 days. Carbon dioxide (CO_2) trapped in the KOH was sampled daily for the first 7 to 10 days and at 2-3 day intervals thereafter. The soil in the reactors was aerated by equilibration with atmospheric air for 20 min. after the CO_2 traps were sampled. Daily and cumulative CO_2 production and percent mineralization of test substance in soil reactors were calculated relative to the negative controls.

Results

Degradation:	Readily biodegradable
Nominal concentrations:	0 (negative control), 0.8, 1.6, 3.2, 6, 12 mg C/g dry soil
Results:	Substrate decay of the test substance reached 166.8, 124.4, 97.2, 97.4 and 72.9% as $ThCO_2$ by Day 42 in test vessels containing 0.8, 1.6, 3.2, 6.0 and 12.0 mg C/g soil, respectively. Total net mineralization of ATBC (expressed as $\%ThCO_2$) varied as a function of substrate

concentration.

Kinetic: The mineralization of ATBC in soil over 42 days was as follows:

Substrate Loading (mg C/g soil)	% $ThCO_2$	Lag (days) [a]	Decay Constant (day^{-1}) [b]	t_{50} (days) [c]	t_{60} (days) [d]
Cellulose (300)	61.6	3.5	0.1028	32.9	32.9
0.8	166.8	1.5	0.0445	8.4	8.4
1.6	124.4	2	0.0570	10.9	10.9
3.2	97.2	3	0.0485	19.9	19.9
6	97.4	3	0.0362	25.8	25.8
12	72.9	4.5	0.0233	57.9	57.9

[a] Time required for test substance mineralization to reach 10% MAX-CO_2.
[b] Based on % decrease in substrate concentration and calculated using the following equation: $y = y_0 + a \exp^{(-bx)}$.
[c] Time required for test substance mineralization to reach 50% $ThCO_2$.
[d] Time required for test substance mineralization to reach 60% $ThCO_2$.

Breakdown Products: Not stated

Remarks: Decay rates decreased as a function of substrate concentration. However, ATBC is considered to be readily biodegradable at concentrations <3.2 mg C/g soil, based on >60% $ThCO_2$ observed within a 10- to 14-day window following the lag.

Conclusions The biodegradability of the test substance has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability: 1B

Remarks: Reliable without restrictions; comparable to guideline study.

References

Farrell, R. E. 2000. An Assessment of the Aerobic Biodegradability of Triethyl Citrate (TEC) and Acetyltri-n-butyl Citrate (ATBC) in Standard, Laboratory-Scale Soil and Compost Environments. Unpublished report by the Dept. of Soil Science, Univ. of Saskatchewan, SK, Canada and NSF-Biodegradable Polymer Research Center, Univ. of Massachusetts-Lowell, MA, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	[in aerobic soil]

3.5 BIODEGRADATION

Test Substance

Identity:	'A4 Special' (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated (99.0%, minimum specification)
Remarks:	

Method

Method/Guideline followed:	Non-specific method (UML-7645) for measuring substance depletion in soil, developed at the NSF-Biodegradable Polymer Research Center.
Test Type:	Aerobic biodegradation in soil.
GLP:	Not stated
Year:	1996
Contact Time:	52 days
Inoculum:	An unspecified commercial compost seed
Remarks:	Four concentrations of the test substance (reported as 40, 80, 160 and 300 mg C) were exposed to the soil under controlled aerobic conditions and maintained at 30±2°C. The test soil was a standard soil mix (1:1:0.1 potting soil:sand:dehydrated cow manure, w:w:w) was seeded with a commercial compost seed (0.5 g per bioreactor) using a *Recycle Compost Maker* (Ringer Corp., Minneapolis, MN). Carbon dioxide production, expressed as a fraction of the measured or theoretical carbon content of the test substance, was measured over a 52-day period. The degree of biodegradability of the test substance was assessed relative to that of a biodegradable control.

Results

Degradation:	Rapidly biodegradable in soil under aerobic conditions.
Results:	Mineralization of the test substance by day 52 reached approximately 128, 125, 90 and 83% $ThCO_2$ for the 40, 80, 160 and 300 mg C-treatments, respectively.
Kinetic:	Not stated.
Breakdown Products:	Not stated.
Remarks:	

Conclusions The biodegradability of the test substance has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability:	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References

Kouloungis, N.R., L. Lebrecque, R.E.Farrell, D.T. Eberiel, R.A. Gross and S.P.McCarthy. 1996. Biodegradability of Citrate Ester Plasticizers. Unpublished Manuscript. Depts. Of Plastics Engineering, Chemistry and Biology, and the NSF-Biodegradable Polymer Research Center, University of Massachusetts-Lowell.

Other Available Reports

Other

Last Changed:	December 8, 2003
Order Number for Sorting:	
Remarks:	

3.5 BIODEGRADATION

Test Substance

Identity:	Acetyltri-n-butyl Citrate; ATBC (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated (99.0%, minimum specification)
Remarks:	

Method

Method/Guideline followed:	ASTM D 5338; ASTM D 5988
Test Type:	Ultimate biodegradation in actively aerated compost
GLP:	Not stated
Year:	2000
Contact Time:	45 days
Inoculum:	Polyseed® BOD seed inoculum (one capsule per reactor)
Remarks:	Yard-waste compost collected from Laughton's Garden Center (Westford, MA) was amended with 1.15 g test substance per 40 g dry compost and placed in 500-mL bioreactors (Erlenmeyer flasks). Reactors were similarly prepared for positive (cellulose) and negative controls. Reactors were prepared in triplicate for each treatment, seeded with Polyseed® BOD seed inoculum (one capsule per reactor) and incubated in a water bath in the dark at temperatures ranging from 35 to 55°C for 45 days. The temperature range simulated the mesophilic start-up, sanitation, thermophilic and mesophilic curing stages of a true, self-heating compost. The system was aerated with humidified, CO_2-free air. The CO_2 traps were generally sampled daily throughout the exposure period, and twice daily on the day of and day following an incubation temperature change. The water content of the compost was maintained at 60±10%. Daily and cumulative CO_2 production and percent mineralization of test substance were calculated relative to the negative controls. A matured (finished) compost was used as the test matrix. In addition to minimizing background levels of CO_2, the matured compost matrix provided a rich source of both thermophilic microorganisms and the organic nutrients needed to support these microorganisms. However, because matured compost is inherently less active than fresh (active) compost, this test should be considered "conservative." This study is intended only to elucidate information relating to the ultimate biodegradability (i.e. mineralization) of ATBC.

Results

Degradation: Ultimately biodegradable

Nominal concentrations: 0 (negative control) and 17.2 mg-C/g dry soil

Results: Mineralization of the test substance reached 37% $ThCO_2$ in 45 days.

Kinetic: The mineralization of ATBC in soil over the exposure period was as follows:

Substrate Loading (mg C/g soil)	% $ThCO_2$	Lag (days) [a]	Decay Constant (day^{-1}) [b]	t_{50} (days) [c]	t_{60} (days) [d]
Cellulose (11.1)	115.6	3.5	0.0337	16.8	21.4
17.2	36.8	2	0.0391	IND	IND

[a] Time required for test substance mineralization to reach 10% MAX-CO_2.
[b] Based on % decrease in substrate concentration and calculated using the following equation: $y = y_0 + a \exp^{(-bx)}$.
[c] Time required for test substance mineralization to reach 50% $ThCO_2$.
[d] Time required for test substance mineralization to reach 60% $ThCO_2$.
IND = *indeterminate.*

Breakdown Products: Not stated

Remarks: Microbial degradation of the test substance in the compost was, in part, retarded at the very high substrate loading used in this test. Further, problems with the aeration system were reported, particularly in the last two weeks of the test. Despite randomization in bioreactor placement, the reduced air supply due to NaOH crystal formation in the air distribution manifold became more significant along the manifold and throughout the exposure period, potentially influencing the results achieved during this study.

Conclusions The biodegradability of the test substance has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability: 1B

Remarks: Reliable without restriction; comparable to guideline study.

References

Farrell, R. E. 2000. An Assessment of the Aerobic Biodegradability of Triethyl Citrate (TEC) and Acetyltri-n-butyl Citrate (ATBC) in Standard, Laboratory-Scale Soil and Compost Environments. Unpublished report by the Dept. of Soil Science, Univ. of Saskatchewan, SK, Canada and NSF-Biodegradable Polymer Research Center, Univ. of Massachusetts-Lowell, MA, USA.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order Number for Sorting:	
Remarks:	[in actively aerated compost]

3.5 BIODEGRADATION

Test Substance

Identity:	Acetyltri-n-butyl Citrate; ATBC (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated (99.0%, minimum specification)
Remarks:	

Method

Method/Guideline followed:	ASTM D 5338; ASTM D 5988
Test Type:	Respirometry test in 'static' compost biometer system (Farrell et al., 2001)
GLP:	Not stated
Year:	2000
Contact Time:	28-44 days
Inoculum:	Compost microorganisms
Remarks:	Compost was amended with 128 or 725 mg test substance per 50 g dry compost and placed in a 1-L bioreactors (1-L Mason jars fitted with Plexiglas compression lids and a Swagelok gas sampling port). Identical bioreactors were prepared as negative controls. Bioreactors were prepared in triplicate for each treatment, stoppered and incubated in the dark at 50±2°C for up to 44 days. Reactors were similarly prepared for negative controls. Reactors were prepared in triplicate for each treatment and control. Carbon dioxide (CO_2) traps were changed at 12- to 72-hour intervals. The soil in the reactors was aerated by equilibration with atmospheric air for 10 to 15 min. after the CO_2 traps were changed and the compost was hand-mixed to preclude development of anaerobic microenvironments. Water content in the compost was monitored by weight every third CO2-trap change; moisture was adjusted by adding appropriate mass of deionized water. Daily and cumulative CO_2 production and percent mineralization of test substance in soil reactors were calculated relative to the negative controls.

Results

Degradation:	Readily biodegradable
Nominal concentrations:	1.9 and 10.8 mg C/g dry soil
Results:	At the higher test concentration, conversion of substrate-C into CO_2 exceeded 50% $ThCO_2$ in about two weeks and 60% $ThCO_2$ in about three weeks. At the lower test

concentration, conversion of substrate-C into CO_2 exceeded 60% $ThCO_2$ within about four days following the lag period. The study was terminated at 28 and 44 days for the lower and higher test concentration reactors, respectively.

Kinetic: The mineralization of ATBC in soil over the exposure period was as follows:

Substrate Loading (mg C/g soil)	% $ThCO_2$	Lag (days) [a]	Decay Constant (day^{-1}) [b]	t_{50} (days) [c]	t_{60} (days) [d]
Cellulose (6.25)	82.6	2	0.0373	12.2	27.5
1.90	87.0	0.5	0.2398	3.5	4.9
10.8	77.6	1.5	0.0722	14.6	20.8

[a] Time required for test substance mineralization to reach 10% MAX-CO_2.
[b] Based on % decrease in substrate concentration and calculated using the following equation: $y = y_0 + a \exp^{(-bx)}$.
[c] Time required for test substance mineralization to reach 50% $ThCO_2$.
[d] Time required for test substance mineralization to reach 60% $ThCO_2$.

Breakdown Products: Not stated

Remarks: The test substance was considered to be readily biodegradable at tested concentrations in the reactor.

Conclusions

The biodegradability of the test substance under static, isothermal composting conditions has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability: 1B

Remarks: Reliable without restriction; comparable to guideline study.

References

Farrell, R. E. 2000. An Assessment of the Aerobic Biodegradability of Triethyl Citrate (TEC) and Acetyltri-n-butyl Citrate (ATBC) in Standard, Laboratory-Scale Soil and Compost Environments. Unpublished report by the Dept. of Soil Science, Univ. of Saskatchewan, SK, Canada and NSF-Biodegradable Polymer Research Center, Univ. of Massachusetts-Lowell, MA, USA.

Other Available Reports

Farrell, R.E., T.J. Adamczyk, D.C. Broe, J.S. Lee, B.L. Briggs, R.A. Gross, S.P. McCarthy, and S. Goodwin. 2001. Biodegradable bags comparative performance study: A multi-tiered approach to evaluating the compostability of plastic materials. Pp. 337-375 In R.A. Gross and C. Scholz (eds.) Biopolymers from Polysaccharides and Agroproteins. ACS Symposium Series 786; American Chemical Society Washington, D.C., USA.

Other

Last Changed:	December 8, 2003
Order Number for Sorting:	
Remarks:	[in passively aerated compost]

3.5 BIODEGRADATION

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/guideline followed:	EPIWIN (v 3.10) BIOWIN Program (v 4.00)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Contact Time:	Not applicable
Inoculum:	Not applicable
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Degradation:	Fast; Readily degradable
Results:	$t_{½}$ (water) = 8.67 d $t_{½}$ (soil) = 8.67 d $t_{½}$ (sediment) = 34.67 d
Kinetic:	Not applicable
Breakdown Products:	Not applicable
Remarks:	

Conclusions The biodegradability of the test substance has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; Biodegradation Probability Program (BIOWIN), Version 4.00; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

Other Available Reports

Other

Last Changed: December 3, 2003
Order Number for Sorting:
Remarks:

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance

Identity:	Citroflex-A4 (O-acetyl-tributyl citrate) (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Acute, flow-through
GLP:	Not stated
Year:	1974
Species/Strain/Supplier:	Mummichogs/*Fundalus heteroclitus*/Not stated
Analytical Monitoring:	Yes, but unsatisfactory recovery using GC.
Exposure Period:	96 hours
Statistical Methods:	Not stated
Remarks:	The study measured the acute toxicity of the test substance to locally obtained mummichogs during a 96-hour exposure period in flow-through tanks. Fish were acclimated to laboratory conditions for several weeks before use in this study. Following 96-hour exposure, observation for mortality and behavioral effects were monitored for an additional 96 hours. Experimental conditions during this study were as follows: flow through tanks delivered 4.8 volume replacements per day; the dilution water was synthetic seawater (25%); the pH ranged from 6.5-7.6; the dissolved oxygen concentration was 70-95% saturation; temperature was maintained at approximately 23 to 24°C; and fish were not fed during the first 96 hours of an extended exposure period. Mummichogs were 3.5 to 8.5 inches in length.

Results

Nominal concentrations (mg/l):	Up to 120
Measured concentrations (mg/l):	Not stated
Unit:	mg/l
Element Value:	96-hour LC_{50} NOEC
Results:	
LC_{50} (96-hour)	= 59 mg/l (nominal)
NOEC (96-hour)	= 10 mg/l (nominal)
Remarks:	Behavioral effects were observed in fish exposed to 50 mg/l of the test substance in less than 48 hours. Some

survival was seen at 100 mg/l after 96 hours of exposure. The fish exposed to low concentrations of the test substance appeared to be more excitable than usual, while activity was greatly reduced in fish exposed to the higher concentrations. Loss of equilibrium was observed and, at higher concentrations, a marked loss of equilibrium and fine fin movements (especially pectoral fins) was observed. Gill motion was irregular and shallow.

Two fish which died during the exposure period were sent to pathology for examination (pathological findings were not reported). Two severely affected fish were transferred from test solutions to fresh water, one of which fully recovered from exposure to a 120 mg/l test solution for 7.5 hours and the other recovered from exposure to an 80 mg/l test solution after 24 hours. All surviving fish reportedly recovered from treatment-related effects within 48 hours of cessation of exposure to the test substance.

Conclusions

Remarks: The acute toxicity of the test substance to mummichogs (*Fundalus heteroclitus*) has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability (Klimisch): 2B
Remarks: Reliable with restrictions; basic data given comparable to guidelines/standards.

References

Foulds, G. 1974. Potential Environmental Impact of Citroflexes. Unpublished report. Pfizer Ecosystems Laboratory, Groton, CT, USA.

Other Available Reports

Other

Last Changed: December 3, 2003
Order number for sorting:
Remarks: [mummichogs, *Fundalus heteroclitus*]

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance

Identity:	Citroflex-A4 (O-acetyl-tributyl citrate) (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Acute, flow-through
GLP:	Not stated
Year:	1974
Species/Strain/Supplier:	Bluegill sunfish/*Lepomis macrochirus*/Not stated
Analytical Monitoring:	Yes, but unsatisfactory recovery using GC.
Exposure Period:	96 hours
Statistical Methods:	Not stated
Remarks:	The study measured the acute toxicity of the test substance to locally obtained bluegill sunfish during a 96-hour exposure period in flow-through systems. Fish were acclimated to laboratory conditions for > 1 month before use in this study. Following 96-hour exposure, observation for mortality and behavioral effects were monitored for an additional 96 hours. Experimental conditions during this study were as follows: flow through tanks delivered 4.8 volume replacements per day; the pH ranged from 6.5-7.6; the dissolved oxygen concentration was 70-95% saturation; temperature was maintained at approximately 23 to 24°C; and fish were not fed during the first 96 hours of an extended exposure period.

Results

Nominal concentrations (mg/l):	Up to 120
Measured concentrations (mg/l):	Not stated
Unit:	mg/l
Element Value:	96-hour LC_{50} NOEC
Statistical Results:	
Result:	
LC_{50} (96-hour)	= 38 - 60 mg/l (nominal)
NOEC (96-hour)	= 10 mg/l (nominal)
Remarks:	The fish exposed to low concentrations of the test substance appeared to be more excitable than usual, while activity was greatly reduced in fish exposed to the

higher concentrations. Loss of equilibrium was observed and, at higher concentrations, a marked loss of equilibrium and fine fin movements was observed. Gill motion was irregular and shallow. All fish reportedly recovered from treatment-related effects within 48 hours of cessation of exposure to the test substance.

Conclusions

Remarks:

The acute toxicity of the test substance to bluegill sunfish (*Lepomis macrochirus)* has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability (Klimisch): 2B

Remarks: Reliable with restrictions; basic data given comparable to guidelines/standards.

References

Foulds, G. 1974. Potential Environmental Impact of Citroflexes. Unpublished report. Pfizer Ecosystems Laboratory, Groton, CT, USA.

Other Available Reports

Other

Last Changed: December 3, 2003

Order number for sorting:

Remarks: [bluegill sunfish, *Lepomis macrochirus*]

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance

Identity:	Citroflex A-4 (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	99.9%
Remarks:	

Method

Method/guideline followed:	U.S. EPA Method 1000.0 (Larval survival and growth test)
Type:	Static-renewal
GLP:	Yes
Year:	2001
Species/Strain/Supplier:	Fathead Minnow (*Pimephales promelas*)/in-house culture
Analytical Monitoring:	HPLC with UV detector
Exposure Period:	7 days
Statistical Methods:	The LC50/EC50 statistic and 95% confidence interval were calculated using the probit and trimmed Spearman-Karber methods. The NOEC and LOEC for survival and dry weights were determined using Toxcalc. Kolmogorov D's homogeneity of variance, was used, when applicable, and a one-way ANOVA followed by Dunnett's test was used to detect statistical differences between treatment and control groups.
Remarks:	The experiment measured the survival and growth of 18-hour old larval fish after 7 days of exposure to the test substance. The nominal concentrations tested included 0.63, 1.3, 2.5, 5.0 and 10 mg/l and a negative (dilution water) control. Treatment vessels were glass beakers containing 250 ml of test solution. Ten fish larvae were added to each of three replicate beakers per treatment and control. Dilution water was aged blended water characterized as having total hardness and alkalinity ranges as $CaCO_3$ of 150mg/l and 152 mg/l, respectively, and specific conductance of 332 µS/cm. Lighting was maintained at an intensity of 536 lux on a 16-hour daylight photoperiod with 30 minute simulated transition periods. Minnows were fed three times daily with newly hatched brine shrimp and rotifers at a rate such that there was a small amount of food left uneaten at the next feeding. Test solutions were renewed daily

by replacing ≥ 90% of each test solution with fresh solutions after removing as much of the feces and uneaten food from the remaining ~10% solution as possible. The expired water was pooled by test concentration for analysis.

All replicates were monitored daily for survival and sublethal effects. Upon test termination, surviving fish were anesthetized and dried (24 h at 60°C), cooled and weighed by replicate. Fish growth was estimated by change in average dry weight per organism.

Dissolved oxygen concentration (DO) and pH were measured in all test solutions at test initiation, renewal (fresh and expired solutions) and test termination. Total hardness, alkalinity and conductivity measurements were made at test initiation. Temperature was measured in expired solutions at renewal and test termination. All fresh and expired test solutions were sampled for analysis of test substance concentration on Days 0 and 6 and Days 1 and 7, respectively.

Results

Nominal concentrations (mg/l): 0, 0.63, 1.3, 2.5, 5.0 and 10 mg/l

Measured concentrations (mg/l): 0, 0.355, 0.606, 1.28, 2.62 and 5.01 mg/l

Nominal Concentration (mg/l)	Test Substance Concentration (mg/l)				Mean Measured Concentration (mg/l)
	Day 0 [a]	Day 1 [b]	Day 6 [a]	Day 7 [b]	
Control	<MQL	<MQL	<MQL	<MQL	<MQL
0.63	0.401	0.380	0.334	0.305	0.355
1.3	0.750	0.521	0.629	0.522	0.606
2.5	1.500	1.280	1.280	1.040	1.28
5.0	2.990	2.760	2.560	2.160	2.62
10	6.050	4.860	5.200	3.920	5.01

[a] Fresh test solution sampled.
[b] Expired test solution sampled.

Unit: mg/l, as active ingredient

Element Value:

Based on survival statistics:

Mean measured value (mg/l)			
	24-hour	**48-hour**	**7-day**
EC_{50}	3.5 (3.4 - 3.7) [a]	2.8 (2.5 - 3.2) [a]	1.9 (1.8 - 2.1) [a]
NOEC	2.62	1.28	1.28
LOEC	5.01	2.62	2.62

[a] (95% confidence interval)

Based on 7-day growth statistics (based on dry weight):

Mean measured value (mg/l)	
EC_{50}	1.4 (0.72 - 2.7) [a]
NOEC	0.355
LOEC	0.606

[a] (95% confidence interval)

Statistical Results:

Remarks:

The mean measured concentrations of the test substance averaged 39 to 64% of the nominal concentrations. Recoveries from quality control fortifications ranged from 67 to 93% of the nominal concentrations. All reported results are based on mean measured concentrations. Undissolved test substance was observed in the primary stock solutions; however, all test solutions appeared clear and colorless throughout the test with no visible particulates, surface films or undissolved test substance.

Fish mortality during the 7-day exposure period ranged from 0% at 0.355 mg/l and 1.28 mg/l to 100% at 5.01 mg/l. Control survival was 97%. Growth of fish surviving the 7-day exposure ranged from 0.2 mg to 1.2 mg. The mean growth per fish in the control was 1.3 mg. Clinical signs through the exposure period included spinal curvature, erratic swimming, discoloration in fish exposed to 2.62 mg/l. All fish in the 10-mg/l group died within the first day of exposure.

Temperature in the test solutions ranged from 24.5 to 25.3°C. Dissolved oxygen concentrations ranged from

7.4 to 8.45 in fresh solutions and 4.9 to 7.13 mg/l in expired solutions. The pH ranged from 7.69 to 8.27 in fresh solutions and from 7.61 to 8.23 in expired solutions.

Conclusions

Remarks: The end point was adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Warbritton, R. and T. Leak. 2001. Larval Survival and Growth Test of Citroflex A-4 to Fathead Minnow, *Pimephales promelas*, Under Static-Renewal Test Conditions. Unpublished report (no. 46862) by ABC Laboratories, Inc. (testing facility), for Mead Corporation (Sponsor).

Other Available Reports

Other

Last Changed: December 8, 2003
Order number for sorting:
Remarks:

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) ECOSAR Program (v 0.99g)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Species/Strain/Supplier:	Fathead minnow (*Pimephales promelas*)
Analytical monitoring:	Not applicable
Exposure period:	96-hour
Statistical methods:	Not applicable
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Nominal concentrations (mg/l):	Not applicable
Measured concentrations (mg/l):	Not applicable
Unit:	mg/l
Element value:	96-hour LC_{50} = 1.669 mg/l
Statistical results:	Not applicable
Remarks:	

Conclusions

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; ECOSAR Version 0.99g; PC-Computer software developed by ECOSAR Program, Risk Assessment Division (7403), Washington, D.C.

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Citroflex A-4 (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	Not stated (99.0%, minimum specification)
Remarks:	

Method

Method/guideline followed:	U.S. EPA's OPPTS 850.1010
Type:	Acute static
GLP:	Yes
Year:	2001
Species/Strain/Supplier:	Water Flea/*Ceriodaphnia dubia*/laboratory culture
Analytical Monitoring:	Yes; HPLC with UV detector.
Exposure Period:	48 hours
Statistical Methods:	The EC_{50} with 95% confidence intervals was calculated using the Trimmed Spearman-Karber method (EC_{50} SAS program ToxCalc).
Remarks:	The experiment measured the 48-hour acute toxicity of the test substance to *Ceriodaphnia dubia* over a 48-hour exposure period. Dilution water was biologically aged laboratory freshwater prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis. The dilution water was characterized as follows: hardness was 130 to 160 mg/l as $CaCO_3$; alkalinity was 156 mg/l as $CaCO_3$; and conductivity was 337 µS/cm. Daphnids were < 24 hours old at the start of the test. Test vessels were 250-ml all glass beakers containing approximately 200 ml of test solution or control (dilution water). Each of six test solution and control were tested in duplicate and each test vessel held 10 daphnids. Test vessels were not aerated during the exposure period. The pH, dissolved oxygen concentration and temperature were measured in all solutions at 0, 24 and 48 hours. At 24 and 48 hours of the test, daphnids were evaluated for immobility and sublethal responses.

Results

Nominal concentrations:	0 (control), 3.9, 7.8, 16, 31, 63, 125 mg/l.
Mean Measured concentrations:	<MQL (control), 3.82, 4.82, 8.70, 15.5, 17.9 and 60.2 mg/l
Unit:	mg/l
Result:	(Based on mean measured test concentrations)
EC_{50} (48-hour):	7.82 mg/l
LC_{50} (48-hour):	Not determined

NOEC (24-hour): 60.2 mg/l
(48-hour): 4.82 mg/l
LOEC (24-hour): >60.2 mg/l
(48-hour): 8.7 mg/l

Remarks: Mortality (as percent immobilization) of the *Ceriodaphnia dubia* after 48 hours exposure to the test substance ranged from 0% at 3.82 mg/l to 100% at test concentrations of 15.5 and 17.9 mg/l. No mortality was observed in the controls. Cumulative mortality (as percent immobilization) were reported as follows:

Mean Measured Concentration (mg/L)	% Immobilization (n=20)	
	24-hr	48-hr
<MQL	0	0
3.82	0	0
4.82	10 [a]	5
8.70	0	65
15.5	35	100
17.9	5	100
60.2	10	95

[a] Two daphnids not found, assumed immobile until 48 hour observations.

The measured concentrations represent 29 to 98% of the nominal concentrations. The controls and test solutions at the three lowest concentrations (≤8.70 mg/l) appear clear, colorless and free of visible particulates, surface film, undissolved test substance and precipitate. Test solutions at the 3 highest concentrations (≥15.5 mg/l) contained undissolved test substance and a surface film.

Water quality parameters remained within acceptable testing limits for *Ceriodaphnia dubia*: temperature was maintained at 25±2°C; dissolved oxygen concentrations ranged from 6.99 to 8.03 mg/l; pH ranged from 7.46 to 8.34; hardness was 154 mg/l as $CaCO_3$; alkalinity was 156 mg/l as $CaCO_3$; and conductivity was 337 µS/cm.

Conclusions The 48-hour acute toxicity of the test substance to *Ceriodaphnia dubia* has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Warbritton, R. and T. Leak. 2001. Acute Toxicity Test of Citroflex A-4 to the Water Flea, *Ceriodaphnia dubia*, Determined under Static Test Conditions. Unpublished report (no. 46891) by ABC Laboratories, Inc. (testing facility), for Mead Corporation (Sponsor), dated December 11, 2001.

Other Available Reports

Other

Last Changed:	December 3, 2003
Order number for sorting:	
Remarks:	

4.2 TOXICITY TO AQUATIC INVERTEBRATES

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) ECOSAR Program (v 0.99g)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Species/Strain/Supplier:	Daphnid (*Daphnia magna*)
Analytical monitoring:	Not applicable
Exposure period:	48-hour
Statistical methods:	Not applicable
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Nominal concentrations (mg/l):	Not applicable
Measured concentrations (mg/l):	Not applicable
Unit:	mg/l
Element value:	48-hour LC_{50} = 0.704 mg/l
Statistical results:	Not applicable
Remarks:	

Conclusions

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; ECOSAR Version 0.99g; PC-Computer software developed by ECOSAR Program, Risk Assessment Division (7403), Washington, D.C.

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

4.3 TOXICITY TO AQUATIC PLANTS (ALGAE)

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not applicable

Method

Method/Guideline followed:	EPIWIN (v 3.10) ECOSAR Program (v 0.99g)
Type:	Not applicable
GLP:	Not applicable
Year:	2003
Species/Strain/Supplier:	Green Algae (*Selenastrum capricornutum)*
Analytical monitoring:	Not applicable
Exposure period:	96-hour
Statistical methods:	Not applicable
Remarks:	The EPIWIN model was run with the following physico-chemical property input values: water solubility of 5 mg/l, vapor pressure of 0.052 mm Hg, log K_{ow} of 4.92, boiling point of 326°C and melting point of –59°C.

Results

Nominal concentrations (mg/l):	Not applicable
Measured concentrations (mg/l):	Not applicable
Unit:	mg/l
Element value:	96-hour EC_{50} = 0.148 mg/l
Statistical results:	Not applicable
Remarks:	

Conclusions

Remarks:	The endpoint has been adequately characterized (Morflex, Inc.).

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; model data.

References

U.S. EPA (Environmental Protection Agency). 2000. EPI Suite™, Version 3.10; ECOSAR Version 0.99g; PC-Computer software developed by ECOSAR Program, Risk Assessment Division (7403), Washington, D.C.

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.1.1 ACUTE ORAL TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Acute oral toxicity
GLP:	No
Year:	1959
Species/Strain:	Rat/strain not stated
Sex:	Not stated
No. of animals:	5
Vehicle:	Not stated
Route of administration:	Oral gavage
Remarks:	A group of five rats were administered by stomach tube a single gavage dose of the test substance at doses of 10 to 30 ml/kg. All animals were observed for signs of toxicity for 21 days following dosing.

Results

Value:	LD_{50} >30 ml/kg
Number of deaths:	No deaths occurred at any dose level throughout the study.
Remarks:	Shortly after administration, the material began to leak from the rectum. Signs of systemic toxicity were not observed; however, transient sluggishness was reported.

Conclusions

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	2A
Remarks:	Reliable with restrictions; acceptable, well-documented publication which meets basic scientific principles.

References

Finkelstein, M and H. Gold. 1959. Toxicology of the Citric Acid Esters: Tributyl Citrate, Acetyl Tributyl Citrate, Triethyl Citrate, and Acetyl Triethyl Citrate. Toxicology and Applied Pharmacology 1:283-298.

Gold, H., W. Modell and M. Finkelstein. 1959. On the pharmacology of triethyl, acetyl triethyl, tributyl, and acetyl tributyl citrates by oral administration in rats and cats. Cornell University Medical College.

Other available reports

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks:

5.1.1 ACUTE ORAL TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Acute oral toxicity
GLP:	No
Year:	1959
Species/Strain:	Cat/strain not stated
Sex:	Not stated
No. of animals:	8
Vehicle:	Not stated
Route of administration:	Oral gavage
Remarks:	After fasting for 24 hours, a group of 4 cats were administered, by stomach tube, a single gavage dose of the test substance at doses of 30 to 50 ml/kg. The animals were observed for periods of up to two months. In addition, two cats were given a single oral dose (via stomach tube) of the test substance at 50 ml/kg. A control group consisting of two cats also was included (control substance not identified in the article). These animals were evaluated at two week intervals during a two month post-dosing period for the following: body weight, red and white blood cell counts, hemoglobin, blood sugar, blood NPN, blood creatinine, and urinalysis (including, specific gravity, albumin, sugar, pH and microscopic examination).

Results

Value:	LD_{50} >50 ml/kg
Number of deaths:	No deaths occurred at any dose level throughout the study.
Remarks:	The animals showed signs of slight nausea and within a few hours developed diarrhea with oozing of the oily material from the rectum. The diarrhea subsided in less than 24 hours. There were no signs of systemic toxicity as judged by the general appearance and behavior of the animals. There were no affects noted on the blood counts, blood chemistry or urinalysis of the treated animals compared to the controls.

Conclusions

Remarks: The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publication which meets basic scientific principles.

References

Finkelstein, M and H. Gold. 1959. Toxicology of the Citric Acid Esters: Tributyl Citrate, Acetyl Tributyl Citrate, Triethyl Citrate, and Acetyl Triethyl Citrate. Toxicology and Applied Pharmacology 1:283-298.

Gold, H., W. Modell and M. Finkelstein. 1959. On the pharmacology of triethyl, acetyl triethyl, tributyl, and acetyl tributyl citrates by oral administration in rats and cats. Cornell University Medical College.

Other available reports

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Test type:	Oral
GLP:	No
Year:	1959
Species:	Rat
Strain:	Not stated
Route of administration:	Oral (feed)
Duration of test:	Six weeks
Doses/concentration levels:	5% and 10%
Sex:	Male and female
Exposure period:	Six weeks
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	None
Statistical methods:	Statistics not performed due to small sample size.
Remarks:	Groups of four, 21-day old male and female rats were fed the test substance at concentrations of 5 and 10% in the diet for six weeks. A third group of four rats was fed the basal diet and served as the control. The appropriate diets were available *ad libitum* to the test and control animals throughout the six-week period. Body weights were measured initially and weekly until study completion.

Results

NOAEL (NOEL)	NOEL = 5%
LOAEL (LOEL)	LOEL = 10%
Actual dose received:	Not stated
Toxic response/effects:	Described below
Statistical results:	None
Remarks:	There were no deleterious effects on growth (as measured by body weight) in the animals provided the diet with 5% test substance. Feeding of the 10% diet resulted in reduced body weight gain, which could have resulted from diarrhea noted in these animals.

Conclusions

Remarks: The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publication/study report which meets basic scientific principles.

References

Finkelstein, M and H. Gold. 1959. Toxicology of the Citric Acid Esters: Tributyl Citrate, Acetyl Tributyl Citrate, Triethyl Citrate, and Acetyl Triethyl Citrate. Toxicology and Applied Pharmacology 1:283-298.

Gold, H., W. Modell and M. Finkelstein. 1959. On the pharmacology of triethyl, acetyl triethyl, tributyl, and acetyl tributyl citrates by oral administration in rats and cats. Cornell University Medical College.

Other available reports

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Test type:	Oral
GLP:	No
Year:	1958
Species:	Rat
Strain:	Not stated
Route of administration:	Oral (feed)
Duration of test:	Eight weeks
Doses/concentration levels:	5% and 10%
Sex:	Male and female
Exposure period:	Eight weeks
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	None
Statistical methods:	Statistics not performed due to small sample size.
Remarks:	Groups of four, 21-day old male and female rats were fed the test substance at concentrations of 5 and 10% in the diet for eight weeks. A third group was fed the basal diet and served as the control. The appropriate diets were available *ad libitum* to the test and control animals throughout the eight-week period. A complete blood count was determined at the start and again after 4 and 8 weeks. At the end of the eight-week feeding period, the all animals were killed and a gross necropsy was performed. The following tissues were also examined microscopically: heart, lungs, gastrointestinal tract, liver, pancreas, spleen, and kidneys.

Results

NOAEL (NOEL)	NOEL = 10%
LOAEL (LOEL)	LOEL = >10%
Actual dose received:	Not stated
Toxic response/effects:	Described below
Statistical results:	None
Remarks:	There were no conspicuous differences in red, white and differential blood counts between the test and control groups. No abnormalities were found at

necropsy or in the organs examined microscopically in either the test or control groups.

Conclusions

Remarks: The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publication/study report which meets basic scientific principles.

References

Finkelstein, M and H. Gold. 1959. Toxicology of the Citric Acid Esters: Tributyl Citrate, Acetyl Tributyl Citrate, Triethyl Citrate, and Acetyl Triethyl Citrate. Toxicology and Applied Pharmacology 1:283-298.

Gold, H., W. Modell and M. Finkelstein. 1959. On the pharmacology of triethyl, acetyl triethyl, tributyl, and acetyl tributyl citrates by oral administration in rats and cats. Cornell University Medical College.

Other available reports

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Test type:	Oral
GLP:	No
Year:	1959
Species:	Cat
Strain:	Not stated
Route of administration:	Oral (gavage)
Duration of test:	Two months
Doses/concentration levels:	5 ml/kg
Sex:	Not stated
Exposure period:	Eight weeks
Frequency of treatment:	Daily
Control group and treatment:	Yes; treatment not stated
Postexposure observation period:	None
Statistical methods:	Statistics not performed due to small sample size.
Remarks:	Two cats received the test substance in doses of 5 ml/kg by stomach tube daily for a period of two months. Each animal received the test substance by gavage at a dose of approximately 50 ml/kg. A control group of two cats also was included in the study design. In addition to observations on general behavior and appearance of the animals, the following parameters were evaluated at weekly intervals throughout the study: body weight, urine, red blood cell counts, white blood cell counts, hemoglobin, blood sugar, blood NPN and blood creatinine.

Results

NOAEL (NOEL)	NOEL < 5 ml/kg
LOAEL (LOEL)	LOEL = 5 ml/kg (based on body weight loss)
Actual dose received:	Not stated
Toxic response/effects:	Described below
Statistical results:	None
Remarks:	Animals in the test substance-treated group developed loose stool and lost weight (presumably due to the loose stool) during the two month dosing period. There was no change in appearance or behavior of the animals and no effect was observed on the urine, blood

chemistry or blood counts.

Conclusions

Remarks: The endpoint has not been adequately characterized; however, this study provides additional information on the toxicity of the test substance after short-term repeated exposure. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publication/study report which meets basic scientific principles.

References

Finkelstein, M and H. Gold. 1959. Toxicology of the Citric Acid Esters: Tributyl Citrate, Acetyl Tributyl Citrate, Triethyl Citrate, and Acetyl Triethyl Citrate. Toxicology and Applied Pharmacology 1:283-298.

Gold, H., W. Modell and M. Finkelstein. 1959. On the pharmacology of triethyl, acetyl triethyl, tributyl, and acetyl tributyl citrates by oral administration in rats and cats. Cornell University Medical College.

Other available reports

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Test type:	Repeated dose
GLP:	No
Year:	1963
Species:	Mouse
Strain:	Not stated
Route of administration:	Intraperitoneal
Duration of test:	14 days
Doses/concentration levels:	900 mg/kg
Sex:	Not stated
Exposure period:	14 days
Frequency of treatment:	Daily
Control group and treatment:	Yes; i.p. injections of vehicle (3% acacia)
Postexposure observation period:	None
Statistical methods:	Not stated
Remarks:	Mice weighed between 16 and 20 grams at study initiation. A group of five mice were given daily i.p. doses of the test substance dissolved in 3% acacia at a concentration of 900 mg/kg. A control group of five mice received daily injections of 3% acacia. The animals were weighed daily and at the end of the two-week dosing period the following parameters were evaluated: red and white blood cell counts, clotting time, and hemoglobin levels. At the end of the 14-day dosing period, two test and two control mice were killed and histopathologic evaluations were performed on the following tissues: liver, lung, and kidneys.

Results

NOAEL (NOEL)	NOEL < 900 mg/kg
LOAEL (LOEL)	LOEL = 900 mg/kg
Actual dose received:	Not stated
Toxic response/effects:	Described below
Statistical results:	Described below
Remarks:	Body weight gain was significantly ($p<0.05$) inhibited compared to the control group beginning 7 days following dose initiation. There were no significant differences in the white blood cell counts, or clotting

times; however, significant ($p<0.05$) decreases in the red blood cell counts and hemoglobin levels occurred in the test animals. There were not significant findings in the tissues examined histologically.

Conclusions

Remarks: The endpoint has not been adequately characterized; however, this study provides additional information on the toxicity of the test substance after short-term repeated exposure. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented publications which meets basic scientific principles.

References

Meyers, D.B., J. Autian and W.L. Guess. 1964. Toxicity of Plastics Used in Medical Practice II. T Toxicity of Citric Acid Esters Used as Plasticizers. Journal of Pharmaceutical Sciences. 53(7):774-777.

Other available reports

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	>98%
Remarks:	

Method

Method/guideline followed:	OECD 408
Test type:	Sub-chronic
GLP:	Yes
Year:	1991
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR)
Route of administration:	Oral (feed)
Duration of test:	90 days
Doses/concentration levels:	0, 100, 300 and 1000 mg/kg/day
Sex:	Male and female
Exposure period:	90 days
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	None
Statistical methods:	Body weights were evaluated by a one way analysis of co-variance (covariate: body weight on Day 0) followed by Dunnett's multiple comparison tests. Food and water consumption, food efficiency, hematology, clinical chemistry, urinalysis, organ weights and hear test (conducted as part of FOB) were evaluated by a one-way ANOVA followed by Dunnett's multiple comparison tests or by Kruskal-Wallis nonparametric ANOVA followed by the Mann-Whitney U-test, as appropriate. Incidence data were evaluated by Fisher's exact probability test. All analyses were two-sided.
Remarks:	*Range-finding study*: In the 14-day range-finding study, 5 rats/sex/dose received the test substance in the diet at concentrations of 0, 1, 2.5 and 5% (w/w) for 14 consecutive days. The test substance intakes at these concentrations were 0, 1000, 2700 and 5000 mg/kg/day, respectively. Body weights, clinical signs, and food consumption were evaluated. At the end of the 14-day treatment period a necropsy was performed on all animals and the following tissues were weighed: brain, heart, kidneys, liver, ovaries, spleen, and testes. The livers of all animals were evaluated microscopically.

90-day sub-chronic study: Based on the results of the 14-day range-finding study, 20 male and 20 female rats (approximately 5 weeks old at study initiation) per group were provided the test substance in the feed at concentrations of 100, 300 and 1000 mg/kg/day for 13 weeks. A control group of 20 rats/sex also was included and received the basal diet. Mortality was checked twice daily and the general condition and behavior of all animals were checked daily. An ophthalmoscopic examination of the cornea, conjunctivae, sclera, iris and fundus oculi of the control and 1000 mg/kg/day rats was made prior to and shortly before termination of treatment. Body weights were measured at study initiation, weekly thereafter and at scheduled necropsy. Food consumption was measured weekly. Food efficiency was calculated and expresses as gram weight gained per gram food consumed. Water consumption was measured for weeks 1 and 11. At approximately week 13, prior to hematology, urinalysis and ophthalmoscopy, a functional observational battery (FOB) was performed on all animals.

On study day 85, blood samples were collected from the tip of the tail of all rats and evaluated for the following parameters: hemoglobin, packed cell volume, red blood cell count, red blood cell distribution width, reticulocytes, total white blood cell count, differential white blood cell count, prothrombin time, thrombocytes, mean platelet volume, platelet distribution width, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration. Clinical chemistry determinations were conducted for all rats shortly before or at the end of the treatment period. On study day 87, blood samples were obtained from the tip of the tail of all rats after fasting from water for 24 hours and food for 16 hours. At necropsy on study days 91 through 94, blood samples were collected from the abdominal aortal of all rats evaluated for the following: alkaline phosphatase activity, aspartate amino transferase activity, alanine aminotransferase activity, gamma glutamyl transferase activity, total protein, albumin, urea, creatinine, bilirubin total, sodium, potassium, calcium, chloride, inorganic phosphate, cholesterol, triglycerides, and phospholipids. On study days 86-87 rats were deprived of water for 24 hours

and of food during the last 16 hours of this period when urine was collected and evaluated for the following parameters: volume, density, appearance, pH, glucose, occult blood, ketones, protein, bilirubin, urobilinogen, microscopy of the sediment. In study week 14, all animals were killed by exsanguinations after anesthesia and examined macroscopically. The standard tissues required by OECD guideline 408, including the reproductive organs (ovaries, oviduct, uterus, vagina, prostate, seminal vesicles/coagulating glands, epididymides, and testes) were retained from all animals and evaluated histopathologically from all control and 1000 mg/kg/day group animals. In addition, the kidneys, liver, lungs and gross lesions were examined for all rats in the 100 and 300 mg/kg/day dose groups. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid with parathyroids.

Results

NOAEL (NOEL)	NOEL = 300 mg/kg
LOAEL (LOEL)	LOEL = 1000 mg/kg
Actual dose received:	Males: 101, 302 and 996 mg/kg day Females: 100, 296 and 999 mg/kg/day
Toxic response/effects:	Described below
Statistical results:	Described below
Remarks:	*Range-finding study*: All animals survived to study termination. Transient dose-related reductions in body weights and food consumption were observed in all test groups; however, body weights at 5000 mg/kg/day in both sexes and in males at 2700 mg/kg/day and food consumption at 5000 mg/kg/day in males remained slightly lower than the corresponding control throughout the study. Microscopic examination of the liver revealed increased cytoplasmic eosinophilia accompanied by reduced glycogen content of periportal hepatocytes in male and females at 5000 mg/kg/day and in two males at 2700 mg/kg/day. There was no evidence of necrosis. *90-day sub-chronic study*: All rats survived to scheduled necropsy and there were no treatment-related clinical signs noted throughout the study. Mean body weights were slightly reduced (although not statistically significant) in both males and females at 1000 mg/kg/day and also in females at

300 mg/kg/day beginning on day 28. Food consumption was slightly reduced in males at 1000 mg/kg/day from day 28 onwards, but food conversion efficiency was not different between treated and control animals. Platelet distribution width and mean platelet volume were increased in both sexes at 1000 mg/kg/day and in males at 300 mg/kg/day; however, without any changes in platelet count or red or white blood cell parameters, these differences were not considered to be treatment-related. Semiquantitative observations revealed a decreased urinary pH in males at 1000 mg/kg/day and fewer crystals in urine at 1000 mg/kg/day in both sexes and in males at 300 mg/kg/day. Increased alkaline phosphatase activity for males in the 1000 mg/kg/day group, decreased fasting glucose for females in the 300 or 1000 mg/kg/day groups, and decreased alanine aminotransferase activity and bilirubin concentration for females in the 1000 mg/kg/day group were measured. In the absence of a consistent pattern for both sexes and lacking any histopathological findings, these differences were considered to be chance findings and unrelated to treatment. Increased relative liver weights for both sexes in the 1000 mg/kg/day group and for males in the 300 mg/kg/day group were not associated with any evidence of hepatotoxicity as evaluated by histopathological examination or clinical chemistry. The only other organ weight change was a slightly increased relative kidney weight for males in the 1000 mg/kg/day group.

Water intake, red blood cell parameters, total and differential white blood cell counts, and renal concentrating ability were unaffected by treatment. In addition, gross necropsy examinations, histopathological examinations, functional observations of appearance, behavior, motor activity, sensory activity or autonomic activity revealed no treatment-related differences.

Conclusions

Remarks: A few statistically significant differences were seen between controls and animals administered 1000 mg/kg/day of ATBC in the diet for 90-days; therefore, a no-observed-adverse-effect level (NOAEL) was established at the 300 mg/kg/day dose

level under the conditions of this study. (Morflex, Inc.) The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Jonker, I.D., V.M.H. Hollanders. 1991. Subchronic (90-day) Dietary Toxicity Study with Acetyl Tributyl Citrate (ATBC) in Rats. Report No. V 91.255. TNO Nutrition and Food Research, The Netherlands.

Jonker, I.D., V.M.H. Hollanders. 1990. Range-Finding Study (14-day, dietary) with Acetyl Tributyl Citrate (ATBC) in Rats. Report No. V 90.335. TNO Nutrition and Food Research, The Netherlands.

Other available reports

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks:

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	99.4%
Remarks:	

Method

Method/guideline followed:	Not stated
Test type:	Oral
GLP:	No
Year:	1950
Species:	Rat
Strain:	Sherman
Route of administration:	Oral (feed)
Duration of test:	Two years
Doses/concentration levels:	0, 200, 2000 or 20,000 ppm
Sex:	Not stated
Exposure period:	Two years
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	None
Statistical methods:	Not stated
Remarks:	Three groups of 20 rats each (approximately 12 weeks of age) received diets containing the test substance (ATBC) at concentrations of 200, 2000 or 20,000 ppm. A group of 40 rats received the basal diet without test substance and served as the control group. Animals had access to their respective diets *ad libitum* for approximately two years. Animals were observed daily for behavior and general condition and body weights were measured weekly. All animals that died spontaneously during the experiment and those surviving to study termination were subjected to a necropsy and tissues were examined histologically. Because of unexplained transient depression of the rate of growth of all three experimental groups from week 5 to 15, two additional groups of 10 rats each received the test substance in the diet at concentrations of 200 and 2000 ppm and one group of 20 rats that served as the control, received the untreated diet for one year. All animals in these additional groups that died spontaneously and those sacrificed at the end of the one year treatment period were studied for pathological changes.

Results

NOAEL (NOEL)	NOAEL = 2000 ppm (estimated as 100 mg/kg/day)
LOAEL (LOEL)	LOAEL = 20,000 ppm (estimated as 1000 mg/kg/day)
Actual dose received:	Estimated as 0, 10, 100 or 1000 mg/kg/day
Toxic response/effects:	Described below
Statistical results:	Described below
Remarks:	In the main study, a transient reduction in body weight gain was observed in animals in all three treated groups, 200, 2000 and 20000 ppm. This decrease in body weight gain was not seen in the additional study of animals treated for one year at dietary concentrations of 200 and 2000 ppm. Since this finding was not reproducible it is considered to be and artifact. Statistical analysis indicated that there were no significant differences between the body weights of the treated animals compared to the concurrent controls. There were no treatment-related clinical observations. Twelve of the 60 rats fed test diets and eight of the 40 control rats died prior to scheduled sacrifice. There was no significant difference in time of death or percentage mortality among the three treated groups and controls. Inflammatory disease of the lungs was the most frequent finding necropsy of these animals, it is likely that this was caused by infection rather than treatment with ATBC. Lymphoid tumors of the pleural and abdominal cavities, with some infiltration of the associated organs, were seen in both treated and control animals at comparable rates and, therefore, were not considered to be treatment-related. Careful examination of the endocrine system did not reveal evidence of abnormality in any of the animals. There were no significant differences between treated and control animals in comparisons of the pathological findings.

Conclusions

Remarks:	Under the conditions of this study, the NOAEL appears to be 20,000 ppm (estimated as 1000 mg/kg/day) ATBC in the diet; however, considering the pre-GLP timing of the study and the lack of experimental detail available in the study report, a more conservative and appropriate NOAEL may be 2000 ppm (estimated as 100 mg/kg/day) ATBC in the diet. (Morflex, Inc.) The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; data are reliable but article lacks details

References

Soeler, A.O., M. Clinton, J. Boggs, and P. Drinker. 1950. Experiments on the Chronic Toxicity of Acetyl Tributyl Citrate. Department of Industrial Hygiene, Harvard Medical School, Boston, MA, USA.

Other available reports

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.4 REPEATED DOSE TOXICITY

Test Substance

Identity:	Citroflex® A-4 (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	99.9%
Remarks:	

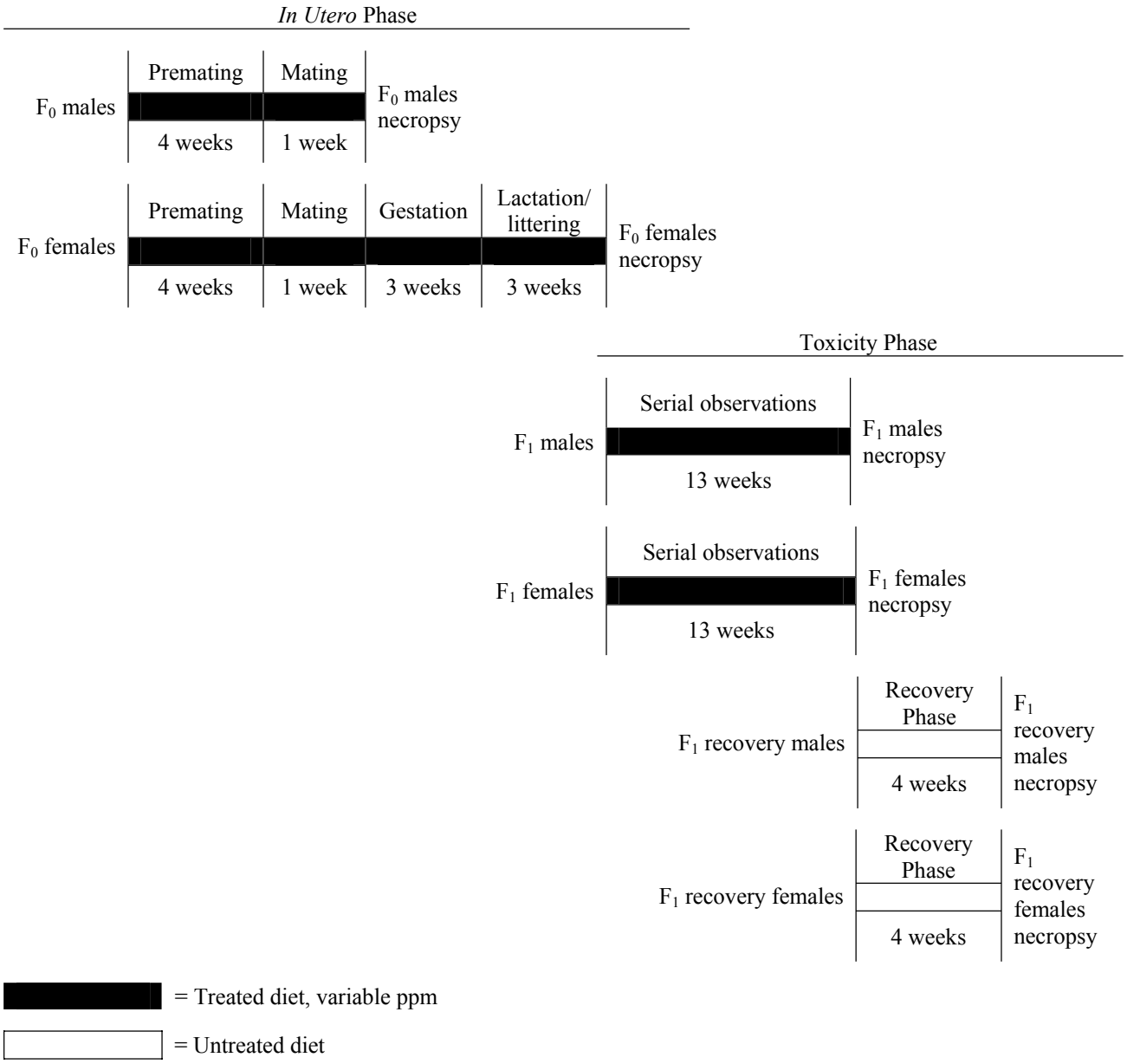
Method

Method/guideline followed:	US EPA OPPTS 870.3100; OECD Method 408; EC Method B26
Test type:	Oral
GLP:	Yes
Year:	2002
Species:	Rat
Strain:	Han Wistar
Route of administration:	Oral (feed)
Duration of test:	F_0 males and females: 4 weeks premating, mating; F_0 females: through gestation and lactation; F_1: 13-weeks plus a 4-week recovery period.
Doses/concentration levels:	0, 100, 300 and 1000 mg/kg/day
Sex:	Males and females
Exposure period:	F_0 males and females were treated for four weeks prior to mating until scheduled sacrifice. The F_1 male and female offspring were exposed *in utero* and from birth until the start of the 13-week study. The F_1 offspring selected for the 13-week study were then provided the respective treated diets for 13-weeks.
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	4 weeks
Statistical methods:	For organ weights and body weight changes, homogeneity of variance was tested using Bartlett's test followed by Behrens-fisher test or Dunnett's test as appropriate. Macroscopic pathology and histopathology data were assessed using Fisher's Exact test. Estrus cycles were analyzed using the Cochran-Armitage trend test. Other statistical tests used as appropriate were: Williams' test for a dose-related response; Student's t-test; Shirley's non-parametric test for a dose-related response; Steel's test; and Wilcoxon rank sum test. Significance level was $p<0.05$.
Remarks:	The study design is shown in Figure 1. Parental (F_0) animals (25 rats/sex/group) were exposed to Citroflex® A-4 (ATBC) continuously in the diet at target doses of 0, 100, 300 and 1000 mg/kg/day for four weeks before

pairing and throughout mating. F_0 males were sacrificed after mating and treatment of the F_0 females continued throughout gestation, littering and lactation until they were killed after the litters were weaned on lactation day 21. Parental males and females were evaluated for reproductive endpoints as discussed in the summary of this study for endpoint 5.8 Toxicity to Reproduction.

The offspring (F_1 generation) were exposed to the test material *in utero* and from birth until the start of the 13-week study (when the animals were approximately four weeks of age). During the 13-week study, the F_1 animals (20/sex/group) were administered ATBC in the diet at the same target doses as the parental animals. An additional 10 F_1 males and 10 F_1 females were assigned to the control and high dose group for a four-week recovery period following the 13-week treatment period.

Study Design for 13-Week Toxicity Study with an *In Utero* Exposure Phase



Results

NOAEL (NOEL)	NOAEL (males) = 100 mg/kg/day NOAEL (females) = 300 mg/kg/day
LOAEL (LOEL)	LOEL (males) = 300 mg/kg/day LOEL (females) = 1000 mg/kg/day
Actual dose received:	103, 306 and 1013 mg/kg/day for males 102, 306 and 1024 mg/kg/day for females
Toxic response/effects:	Described below
Statistical results:	Described below
Remarks:	The results of the *in utero* phase are discussed in the summary of this study for endpoint 5.8 Toxicity to Reproduction.

At the completion of the *in utero* phase, rats that had been exposed to ATBC from before conception, through gestation and continuously from the time of birth were selected (20 unrelated males and 20 unrelated females per dose group for the main study; and 10 unrelated males and 10 unrelated females for the control and high dose recovery groups) and transferred to the 13-week study. There were no significant intergroup differences in the body weights of the animals at the start of the 13-week study.

In the 13-week toxicity phase of the study, administration of ATBC via the diet to Han Wistar rats at doses as high as 1000 mg/kg/day that had already received direct and indirect exposure to the test material from before conception did not produce any marked toxicity. Treatment at 1000 mg/kg/day resulted in a slight reduction in body weight gain in both sexes, which was considered to be a nonspecific indicator of toxicity. Liver weights were increased and hepatic hypertrophy occurred at 1000 mg/kg/day in both sexes. Hepatic hypertrophy resulting from an induction of metabolizing enzymes as an adaptive response to treatment is a common finding following administration of high doses of xenobiotics, and is not considered to be toxicologically significant. Weak peroxisome proliferation was measured in males at 300 mg/kg/day and both sexes at 1000 mg/kg/day. Peroxisome proliferation is universally recognized as a rodent specific effect and not relevant to hazard characterization for humans. Slight variations in urinary composition and in plasma electrolyte concentrations suggested an effect on renal function at

the higher dose levels. In view of the slight nature of these changes, which were all shown to be reversible and within normal historical control ranges, and the lack of histopathological changes in the kidneys, the possible effect on renal function is considered to be due to adaptation to the excretion of high levels of the test material and/or metabolites and is not considered to be of any toxicological significance.

Conclusions

Remarks: The no-observed-adverse-effect levels (NOAELs) for systemic toxicity for ATBC in this 13-week toxicity study with an *in utero* exposure phase were considered to be 100 mg/kg/day for males and 300 mg/kg/day for females. (Authors of report)
The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; modified guideline study

References

Chase, K. R. and C. R. Willoughby. 2002. Citroflex® A-4 Toxicity Study by Dietary Administration to Han Wistar Rats for 13 Weeks with an *In Utero* Exposure Phase Followed by a 4-Week Recovery Period. Project No. MOX 002/013180. Huntingdon Life Sciences Ltd., UK.

Other available reports

Other

Last changed: December 9, 2003
Order number for sorting:
Remarks:

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	98.55%
Remarks:	

Method

Method/guideline followed:	OECD 471
Type:	Reverse mutation assay
System of testing:	Bacterial
GLP:	Yes
Year:	1988
Species/Strain:	Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537
Metabolic activation:	With and without metabolic activation; Aroclor 1254-induced rat liver S-9 from Sprague-Dawley rats
Concentrations tested:	50, 158, 500, 1580 and 5000 µg per plate.
Statistical methods:	Not stated
Remarks:	An initial assay at concentrations of 5, 15.8, 50, 158, 500, 1580 and 5000 µg/plate was conducted with TA100 to assess the cytotoxicity of the test substance. Based on the results of this assay the top five concentrations were chosen for the mutagenicity assay. The test substance was diluted in DMSO, which also was used as the vehicle control. The pre-incubation assay was employed to optimize the contact between the bacteria and test chemical. The pre-incubation assay was also used for all negative and positive controls. Two independent experiments were conducted with each strain. 2-Anthramine (2.0 µg/plate) was the positive control for all tester strains with S-9 activation. The positive controls utilized without S-9 activation were as follows: 2-nitrofluorene (100 µg/plate, TA98); sodium azide (25.0 µg/plate, TA100 and TA1535); and ICR-191 (10 µg/plate, TA1537). All positive controls were prepared in DMSO expect for sodium azide which was prepared in distilled water. The positive control, vehicle control and all test substance doses were plated in triplicate. All strains were tested both with and without metabolic activation (S-9). The S-9 rat liver homogenate was obtained from an outside supplier.

Results

Result:	The test substance showed no evidence of mutagenic activity when tested in this bacterial system with and without activation.
Cytotoxic concentration:	Not cytotoxic up to 5000 µg/plate
Genotoxic effects:	Negative with and without S-9 activation
Statistical results:	Not stated
Remarks:	None

Conclusions

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	1A
Remarks:	Reliable without restriction; guideline study.

References

Gollapudi, B.B. and V.A. Linscombe. 1988. Evaluation of Acetyl Tributyl Citrate in the Ames Salmonella/mammalian-Microsome Bacterial Mutagenicity Assay. Health and Environmental Services, Texas, USA.

Other available reports

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Ames, B.N., J. McCann and E. Yamasaki. 1975. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test, Mutation Research, 31:347-364.
Type:	Reverse mutation assay
System of testing:	Bacterial
GLP:	Yes
Year:	1991
Species/Strain:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic activation:	With and without metabolic activation; liver S-9 from Aroclor 1254-induced Sprague-Dawley rats and Syrian Golden Hamsters
Concentrations tested:	333, 1000, 3333, 6667 and 10,000 µg per plate.
Statistical methods:	Not stated
Remarks:	The plate incorporation method was employed for both the cytotoxicity and mutagenicity assays. An initial assay with 10 concentrations up to 10,000 µg/plate was conducted with TA100 to assess the cytotoxicity of the test substance. Based on the results of this assay the top five concentrations were chosen for the mutagenicity assay. The test substance was diluted in DMSO, which also was used as the vehicle control. 2-Aminoanthracene (1.0 µg/plate) was the positive control for all tester strains with S-9 activation. The positive controls utilized without S-9 activation were as follows: 2-nitrofluorene (1 µg/plate, TA98 and TA1538); sodium azide (1.0 µg/plate, TA100 and TA1535); and 9-aminoacridine (75 µg/plate, TA1537). The positive controls, vehicle control and all test substance doses were plated in triplicate. All strains were tested both with metabolic activation from rat S-9 and hamster S9. A confirmatory assay with DMSO as the solvent was not performed.

Results

Result:	The test substance showed no evidence of mutagenic activity when tested in this bacterial system in the presence of both rat and hamster liver S-9 and in the absence of microsomal activation.
Cytotoxic concentration:	Not cytotoxic up to 10,000 µg/plate
Genotoxic effects:	Negative with and without S-9 activation
Statistical results:	Not stated
Remarks:	Slight precipitate was observed at concentrations from 1000 µg/plate and higher

Conclusions

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restriction (a confirmatory assay was not conducted).

References

San, R.H.C. and V.O. Wagner. 1991. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test). Laboratory Study Number C316.501017. Microbiological Associates, Inc., Rockville, MD, USA.

Other available reports

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl-tributylcitrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	The test substance was extracted from plastic film for testing

Method

Method/guideline followed:	Ames, B.N., J. McCann and E. Yamasaki. 1975. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test, Mutation Research, 31:347-364.
Type:	Reverse mutation assay
System of testing:	Bacterial
GLP:	Yes
Year:	1982
Species/Strain:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic activation:	No
Concentrations tested:	9, 50, 99 and 495 µg
Statistical methods:	Not stated
Remarks:	The plate incorporation method was employed in this mutagenicity assay. Nitrofluorene was used as the positive control for all tester strains. The test substance was diluted in DMSO. An untreated control group also was included.

Results

Result:	The test substance showed no evidence of mutagenic activity when tested in this bacterial system in the absence of microsomal activation.
Cytotoxic concentration:	Not cytotoxic up to 495 µg
Genotoxic effects:	Negative without metabolic activation
Statistical results:	Not stated
Remarks:	Tester strains TA1538 and TA1535 were not sensitive to the positive control used in this experiment. The test substance was not cytotoxic to any tester strains at concentrations up to 495 µg.

Conclusions

Remarks:	The endpoint has not been adequately characterized; however, the information in this article confirms the results of other studies. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; minimal test conditions were evaluated and a confirmatory assay was not conducted.

References

Heath, J.L. and M. Reilly. 1982. Mutagenesis Testing of Acetyl-Tributylcitrate and Epoxidized Soybean Oil. Poultry Science, 61:2517-2519.

Other available reports

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	98.55%
Remarks:	

Method

Method/guideline followed:	OECD 473
Type:	Cytogenetic assay (chromosomal aberration)
System of testing:	Nonbacterial (rat lymphocytes)
GLP:	Yes
Year:	1988
Species/Strain:	Rat/Sprague-Dawley (Crl:CD BR)
Metabolic activation:	With and without S-9 activation; S-9 mix obtained from the liver of Aroclor 1254-induced male Sprague-Dawley rats
Concentrations tested:	4, 13.3, 40, 133 and 400 µg/ml (both in the presence and absence of S-9)
Statistical methods:	Chi-square analysis using a 2 x 2 contingency table
Remarks:	Cultures were initiated by inoculating approximately 0.3 ml of whole blood/5 ml of culture medium. Cultures were set up in duplicate at each dose level and incubated at 37°C. Forty-eight hours after setting up of whole blood cultures, the rat lymphocytes were treated with the test substance for four hours at concentrations of 0 (negative control), 4, 13.3, 40, 133 and 400 µg/ml both with and without S-9 metabolic activation. S-9 liver homogenate prepared from Aroclor 1254-teated Sprague-Dawley rats was purchased from an outside vendor. S-9 mix was prepared fresh and used on the day of preparation. DMSO was used to dissolve the test substance and also was used as the negative control group substance. The final concentration of DMSO in the culture medium was 1%. Cultures treated with 1000 µg/ml ethyl methanesulfonate and 4.2 µg/ml cyclophosphamide were used as positive controls for the non-activation and activation assays, respectively. The cultures were harvested 24 hours after termination of treatment. Mitotic indices were determined as the number of cells in metaphase among 1000 cells/replicate and expressed as percentages. Based upon the data on mitotic indices, cultures treated with the test substance at concentrations of 50, 133 and 400 µg/ml were selected for determining the frequencies of chromosomal

aberrations. A total of 100 cells were scored in each replicate in the treated and negative control cultures and 50 cells were scored in the positive control cultures. Only those metaphases that contained 42 chromosomes were scored with the exception of severely damaged cells (cells having ≥10 aberrations/cell), in which case, accurate counts of the chromosomes were not possible. Gaps were not included in calculations of total cytogenetic aberrations. The following aberrations were included in the evaluation: chromatid breaks, chromatid exchanges, chromosome breaks, and chromosome exchanges.

Results

Result:	The test substance showed no evidence of mutagenic activity in the presence and absence of an S-9 metabolic activation system.
Cytotoxic concentration:	None
Genotoxic effects:	Negative with and without metabolic activation
Statistical results:	Described below
Remarks:	In preliminary solubility trials, the test material was found to be insoluble in the culture medium at concentrations from 667 to 3333 µg/ml. At concentrations of 333 µg/ml and below, the test substance was uniformly suspended in the culture medium.

The frequencies of cells with aberrations in the test substance-treated cultures ranged from 1% to 3% in the absence of S-9 and 1.5% to 3.5% in the presence of S-9 activation. These values were neither significantly different from the concurrent controls nor outside the historical range. Significant increased in aberration rates were observed in the positive controls both with and without S-9 activation.

The average mitotic index and average total aberrations are provided in the following table:

Dose Group	S-9	Mitotic Index (%)	No. (%) of Cells with Aberrations
Negative control	–	6.5	4 (2.0)
	+	3.1	3 (1.5)
40 µg/ml	–	3.4	6 (3.0)
	+	4.3	3 (1.5)
133 µg/ml	–	5.9	2 (1.0)
	+	4.8	4 (2.0)
400 µg/ml	–	5.4	2 (1.0)
	+	4.4	7 (3.5)
Positive control	–	2.0	28* (28.0)
	+	2.8	38* (38.0)

+ = With S-9 activation.
– = Without S-9 activation.
* Significantly different ($\alpha \leq 0.01$) from the negative control.

Conclusions

Remarks: Under the conditions of the assay described in this report, the test substance did not produce clastogenic response in cultured rat lymphocytes. (Author of report).

The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Dow Chemical Company. 1988. Evaluation of Acetyl Tributyl Citrate in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes. Sanitized Laboratory Report. [Name of Testing Facility not stated].

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks:

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	*In vivo/in vitro* unscheduled DNA synthesis
System of testing:	Hepatocyte primary cell culture
GLP:	Not stated
Year:	1999
Species/Strain:	Han Wistar
Metabolic activation:	Not applicable
Concentrations tested:	800 or 2000 mg/kg
Statistical methods:	Not stated
Remarks:	An acute dose-range finding toxicity study with three male Han Wistar rats indicated that a maximum of 2000 mg/kg could be used for the unscheduled DNA synthesis (UDS) assay. A lower dose of 800 mg/kg was also selected. Groups of five male rats were treated once with the solvent corn oil, the test substance (at 800 or 2000 mg/kg) or the required positive control, by oral gavage at a dose volume of 10 ml/kg. The positive controls used were 75 mg/kg 2-acetamindofluorene (2-AAF) suspended in corn oil (12-14 hour experiment) and 10 mg/kg dimethylnitrosamine (DMN) dissolved in purified water (2-4 hour experiment). Approximately 12-14 hours or 2-4 hours after dosing, animals were killed and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from three animals in each dose group and were treated with [^{3}H] thymadine. Six slides from each animal were made, three of which were processed. Two slides from each animal and dose group were examined microscopically and the net grain count, and the number of grains present in the nucleus minus the mean number of grains in three equivalent areas of the cytoplasm was determined.

Results

Result:	No clinical signs of toxicity were observed in any main study animal. Negative (vehicle) control group

	animals gave a group mean net grain count (NGC) value of less than zero with only 0 to 0.3% cells in repair. Treatment with the test substance at doses of 800 or 2000 mg/kg did not produce group mean NNG values greater than -1.3 nor were there any more than 0.7% cells found in repair at either dose. The responses of the negative and positive controls indicated the assay was valid.
Cytotoxic concentration:	Not stated
Genotoxic effects:	Negative
Statistical results:	Not stated
Remarks:	None

Conclusions

Remarks:	This test substance did not induce unscheduled DNA synthesis in freshly prepared primary cultures of hepatocytes from rats dosed at up to 2000 mg/kg under the conditions employed in this assay. (Morflex, Inc.) The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restrictions.

References

Fellows, M. 1999. Acetyl Tributyl Citrate (ATBC): Measurement of Unscheduled DNA Synthesis in Rat Liver Using an *In Vivo/In Vitro* Procedure. Report No. 1734/1-DE140. Covance Laboratories Limited, North Yorkshire, England.

Other

Last changed/Initials:	December 9, 2003
Order number for sorting:	
Remarks:	

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Mammalian cell forward mutation assay
System of testing:	Nonbacterial
GLP:	Yes
Year:	1991
Species/Strain:	L5178Y TK+/- mouse lymphoma cells
Metabolic activation:	With and without S-9 activation; S-9 mix obtained from the liver of Aroclor-induced rats
Concentrations tested:	1st Initial toxicity assay: 0.1 to 5140 µg/ml (with and without S-9 activation) Mutagenicity assay: 10, 70, 150 and 230 µg/ml (absence of S-9) 200, 270, 340, 410 and 480 µg/ml (presence of S-9)
Statistical methods:	Not stated
Remarks:	Based on the results of the initial toxicity assay, cultures were exposed to the test article over a range of concentrations from 10 to 550 µg/ml for the nonactivated cultures and from 54 to 550 µg/ml for the S-9 activated cultures. After a two-day expression period, two cultures per concentration were selected for cloning based upon their degree of toxicity. Four concentrations (10, 70, 150 and 230 µg/ml) of the nonactivated cultures and five concentrations (200, 270, 340, 410 and 480 µg/ml) of the S-9 activated cultures were evaluated. Solvent and positive controls were also included in the mutagenicity assay. DMSO was the solvent used and the positive control substances were ethyl methanesulfonate and 3-methylcholanthrene for the nonactivated and activated cultures, respectively.

Results

Result:	The test substance showed no evidence of mutagenic activity when tested in this mammalian cell gene mutation assay both with and without metabolic activation.
Cytotoxic concentration:	Complete toxicity was observed during the initial toxicity test at concentrations of 514 µg/ml and above for

nonactivated cultures and from 1028 µg/ml and above for the S-9 activated cultures. A dose-dependent increase in toxicity was observed in the mutagenicity assay, with an average Total Growth of 16%, 6% and 3% in the nonactivated cultures at concentrations of 70, 150, and 230 µg/ml, respectively with complete toxicity at 310 µg/ml and above. For the S-9 activated cultures, the average Total Growth was 16% and 8% at 410 and 480 µg/ml, respectively, with complete toxicity at 550 µg/ml.

Genotoxic effects: Negative with and without metabolic activation

Statistical results: Described below

Remarks: The mutant frequencies of the cultures treated with the test substance in the absence of metabolic activation ranged from 2.5 to 1.0 times the mean mutant frequency of the solvent controls. However, no cultures with ≥10% total growth exhibited a two-fold increase in mutant frequency over the average solvent control mutant frequency required for a positive result. Although there was a dose-depended increase in cytotoxicity (total growth of these cultures ranged from 3% to 89%), there was not a dose-related increase in mutant frequency. The frequency of small colonies was comparable to the solvent controls in these treated cultures. The mutant frequencies of the cultures treated with the test article in the presence of S-9 activation ranged from 1.2 to 0.6 times the mean mutant frequency of the solvent controls. There was a dose-dependent increase in cytotoxicity, but there was not a dose-related increase in mutant frequency. There was not an increase in the frequency of small colonies when the treated cultures were compared to the solvent control cultures. The results from the solvent and positive controls fulfilled the requirements for a valid test.

Conclusions

Remarks: Under the conditions of this test, the test substance produced a negative response in both the absence and presence of exogenous metabolic activation (Author of report).
The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks:	Reliable with restrictions; acceptable, well-documented study report which meets basic scientific principles.

References

Bigger, C. A. H. and J. W. Harbell. 1991. Mouse Lymphoma Assay (L5178Y TK +/-). Study No. C316.703. Microbiological Associates, Inc. , Bethesda, MD, USA.

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.5 GENETIC TOXICITY *IN VITRO*

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	99.02%
Remarks:	

Method

Method/guideline followed:	EEC *In Vitro* Mammalian Cell Gene Mutation Test, (Official Journal of the European Communities, L133); US EPA Health Effects Testing Guidelines, 40 CFR Part 798; OECD Guidelines for Testing of Chemicals
Type:	Mammalian cell forward mutation assay (HGPRT gene mutation)
System of testing:	Mammalian cells
GLP:	Yes
Year:	1991
Species/Strain:	Chinese hamster ovary cells
Metabolic activation:	With and without S-9 activation; S-9 liver homogenates were prepared from Aroclor 1254-induced male Sprague-Dawley rats
Concentrations tested:	25, 50, 100, 200 and 400 µg/ml
Statistical methods:	The frequencies of mutant per 10^6 clonable cells were analyzed with a pairwise t-test ($p<0.05$), with the pairs being the replications for each dose. In the pairwise analysis, the square roots of the frequencies were used to correct for heterogeneity of variance. Comparisons to the negative control were made with an approximate Chi-square statistic ($p<0.01$, one-sided). Examination of trends was conducted with least squares regression ($p<0.025$, one-sided).
Remarks:	The solvent, DMSO, was used to dissolve the test substance and also was used as the negative control substance in all assays. In a preliminary trial, treatment solutions containing 400 µg/ml of the test substance were classified as "uniform hazy solutions" and, therefore, this concentration was selected as the highest dose for all subsequent experiments. Toxicity assay: Three cultures/dose level were treated with the test substance at concentrations of 25, 50, 100, 200 and 400 µg/ml in the presence and absence of S-9 factor. After termination of treatment, cultures were incubated for up to 7 days to allow colony formation, fixed with methanol and stained with crystal violet. The number of colonies/plate was counted and the mean colonies/plate/treatment were expressed relative

to the negative control.
Gene Mutation Assay: Each dose level was set up in duplicate from the time of treatment until the completion of the assay. The number of cells to be treated at each dose level was adjusted to yield at least 1 x 10^6 surviving cells. The cultures were trypsinized 18-24 hours after termination of treatment and replated. During the phenotypic expressions period, the cells were cultured once on Day 3 and again on Day 6 and plated at a density of about 1 x 10^6 cells/100 mm dish. On Day 8, the cultures were trypsinized and plated at a density of 2 x 10^5 cells/100 mm dish in the selection media for the determination of $HGPRT^-$ mutants and 200 cells/60 mm dish (5 plates/treatment) for determination of cloning efficiency. The plates were incubated for about 7-9 days, fixed with methanol and stained with crystal violet. The mutation frequency (expressed as mutant per 10^6 clonable cells) at each dose level was calculated by the following formula:

$$\frac{\text{Total number of mutant colonies}/10^5 \text{ plated cells}}{\text{Cloning efficiency}}$$

Where cloning efficiency is defined as:

$$\frac{\text{No. of colonies}}{\text{No. of cells plated}}$$

A confirmatory mutagenicity assay was conducted.

Results

Result:	The test substance showed no evidence of mutagenic activity when tested in this mammalian cell gene mutation assay.
Cytotoxic concentration:	Described below
Genotoxic effects:	Negative with and without metabolic activation
Statistical results:	Described below

Remarks:

The following table summarized the relative cell survival (RCS) data from the initial toxicity assay:

Dose Group	RCS (%)	
	Without S-9	With S-9
Negative control	100	100
25 µg/ml	68.5	87.4
50 µg/ml	50.0	82.3
100 µg/ml	38.2	93.8
200 µg/ml	40.3	86.7
400 µg/ml	36.4	48.5

In the mutation assay precipitate was observed at concentrations of 100, 200 and 400 µg/ml. The following table summarized the mutation frequencies of the mutagenicity assay:

Dose Group	Assay Number	Mutation Frequencies	
		Without S-9	With S-9
Negative control	1	4.363	5.128
	2	3.615	4.311
25 µg/ml	1	7.070	0.000
	2	5.490	24.242
50 µg/ml	1	0.000	5.576
	2	1.821	5.808
100 µg/ml	1	5.968	0.873
	2	2.481	13.675
200 µg/ml	1	13.212	2.657
	2	4.949	24.719*
400 µg/ml	1	10.619	0.000
	2	4.164	1.779
Positive control	1	393.414*	206.621*
	2	439.430*	193.489*

* Significantly different from negative controls (p = 0.001).

The statistically significant increase in mutation frequency observed in the 200 µg/ml concentration seen in assay 2 was interpreted by the study report authors as a chance occurrence unrelated to the treatment because of the following reasons:
1. The response at 200 µg/ml was not dose-related and statistical analysis indicated no significant

dose-response. In fact, the response at 200 µg/ml (24.719) was very similar to the mutation frequency of cultures treated with the lowest concentration of 25 µg/ml (24.242), which was not statistically significant.
2. The mutation frequency of 24.719 was within the range of the laboratory's historical background data.
3. The statistical increase was observed only in one of the two assays performed and, therefore, was not considered to be a reproducible effect.

Conclusions

Remarks: Based upon the results of the experiments, it was concluded that the test substance did not induce a mutagenic response in the CHO/HGPRT mutation assay in the presence and absence of S-9 activation system (Author of report).
The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; guideline study.

References

Dow Chemical Company. 1991. Evaluation of Acetyl Tributyl Citrate in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay. Sanitized Laboratory Report. [Name of Testing Facility not stated].

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks:

5.8 TOXICITY TO REPRODUCTION

Test Substance

Identity:	Acetyl tributyl citrate; Citroflex A-4; and Uniplex 84 (CAS RN 77-90-7)
Purity:	99.4%
Remarks:	The test substance was received from four different suppliers (one batch from each supplier). Equal quantities of each batch were blended together for use on the study.

Method

Method/guideline followed:	Not stated
Type:	Oral; 2-generation reproduction
GLP:	Yes
Year:	1993
Species:	Rat
Strain:	Sprague Dawley
Route of Administration:	Oral (feed)
Doses/concentration levels:	0, 100, 300 and 1000 mg/kg/day
Sex:	Male and female
Control group and treatment:	Yes, untreated diet
Frequency of treatment:	Continually, *ad libitum*
Duration of test:	F_0 males: 11 weeks prior to mating and continued until the day prior to scheduled sacrifice. F_0 females: 3 weeks prior to mating and continued through mating, gestation and lactation period until the day prior to scheduled sacrifice. F_1 males: From weaning, 10 weeks prior mating until the day prior to scheduled sacrifice. F_1 females: From weaning, 10 weeks prior to mating through mating, gestation, lactation until the day prior to scheduled sacrifice.
Premating exposure period for males:	Eleven weeks for F_0 males and at least 10 weeks for F_1 males
Premating exposure period for females:	Three weeks for F_0 females and at least 3 weeks for F_1 females
Statistical methods:	Continuous variable data were tested for normality using the Kolmogorov-Smirnov test ($p<0.05$) and homogeneity of variance using Bartlett's test ($p<0.01$). If both tests were non-significant, the control and test groups were compared using analysis of variance followed by the least significant difference test. If either test was significant, a suitable transformation

test was attempted. Mann-Whitney test or t-test was used when appropriate. In all tests, a probability level of $p<0.05$ in a two sided test was taken to indicate statistical significance.

Remarks:

Thirty males and 30 females per group were provided the test substance (ATBC) in the diet at concentrations of 100, 300 or 1000 mg/kg/day. A control group of 30 animals per sex received the untreated test diet. All animals were housed two per cage by sex except for the mating gestation and lactation periods. After the prebreed exposure period, one male and one female within each dose group were caged together until a sign of mating was observed, for a maximum 14-day mating period. Exposure to the test substance or vehicle continued through mating for males and females, and continued through gestation and lactation for females. Animals were observed daily with a detailed observation being performed weekly. Male rats were weighed daily for three days before and three days after the first day of treatment and then weekly until scheduled sacrifice. Female rats were weighed daily for three days before and three days after the first day of treatment, then weekly for premating and mating periods. During gestation and lactation, body weights were measured daily for the females and then after weaning of the litters, the females were weighed weekly until scheduled sacrifice.

Selected F_1 males and females were weighed weekly after weaning through prebreed and mating periods. Males continued weekly collection of body weights until scheduled sacrifice. Collection of body weights for F_1 females following successful mating followed same schedule as the F_0 females. After parturition (postnatal day 0), total litter weights were obtained for all F_1 and F_2 litters on postnatal days 1, 4, and 14 and individual pup weights were obtained at weaning on day 21. All F_1 and F_2 litters were randomly culled to a maximum of 10 pups per litter on day 4 of lactation. A gross necropsy was conducted on all parental animals sacrificed at scheduled termination, on moribund animals, and on spontaneous deaths. The rats were fasted overnight prior to scheduled necropsy. A full range of tissues were retained. Tissues found to be abnormal at necropsy were evaluated

histopathologically. One weanling per sex from each litter, if available, was subjected to a complete necropsy (including tissue retention as per the parental animals) weaning 14 days of weaning. The remaining weanlings (except those F_1 weanlings selected for parents of the next generation) were killed and examined macroscopically. Diets were analyzed periodically using gas chromatography.

Results

NOAEL: Parental NOAEL = 100 mg/kg/day
Offspring NOAEL = 100 mg/kg/day

Actual dose received: The following tables provide the mean calculated intake of the test substance (mg/kg/day) for the F_0 and F_1 male and female rats:

Dose Group (mg/kg/day)	F_0 Generation	
	Males	**Females**
0	0	0
100	108.8	108.8
300	316.2	363.0
1000	1069.1	1119.1

Dose Group (mg/kg/day)	F_1 Generation	
	Males	**Females**
0	0	0
100	92.8	115.4
300	291.5	321.1
1000	985.8	1113.5

Diet analysis: The analysis of the dietary concentrations indicated that the mean concentration were within 10% of the intended concentrations.

F_0 and F_1 adult data: No treatment-related clinical observations were noted throughout the study in either F_0 or F_1 parental animals. Body weights of F_0 parents and F_1 females were largely unaffected by treatment with ATBC; however, body weights of the F_1 parental males in the 300 and 1000 mg/kg/day groups were consistently lower that controls and appeared to be related to treatment. Body weights of the F_0 females in the 1000 mg/kg/day group at the end of pregnancy (gestation days 21 or 22) was significantly lower than control values. Water consumption of the F_0 and F_1 parental animals fed ATBC at a level of

	1000 mg/kg/day were consistently lower than concurrent controls throughout the study. Mating, gestation and fertility of the F_0 and F_1 generations were unaffected by treatment. There were no abnormalities seen at necropsy that were considered to be treatment-related.
Offspring toxicity:	The body weights of the pups from the 300 and 1000 mg/kg/day dose groups were slightly lower than those of the controls, and slightly higher mortality also was observed in these groups. It was considered that these effects are a consequence of the reduced water intakes in the dams at these dose levels rather than a direct effect of ATBC. No other treatment-related effects were observed in the parameters evaluated.
Statistical results:	Described above
Remarks:	None

Conclusions:

Remarks:	The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability:	2C
Remarks:	Reliable with restrictions; comparable to guideline study with acceptable restrictions (limited histopathologic evaluation).

References

Robbins, M.C. 1994. A Two-Generation Reproduction Study with Acetyl Tributyl Citrate in Rats. Report No. 1298/1/2/94. BIBRA Toxicology International, Surrey, UK.

Other available reports:

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.8 TOXICITY TO REPRODUCTION

Test Substance

Identity:	Citroflex® A-4 (CAS RN 77-90-7; Acetyl tributyl citrate)
Purity:	99.9%
Remarks:	

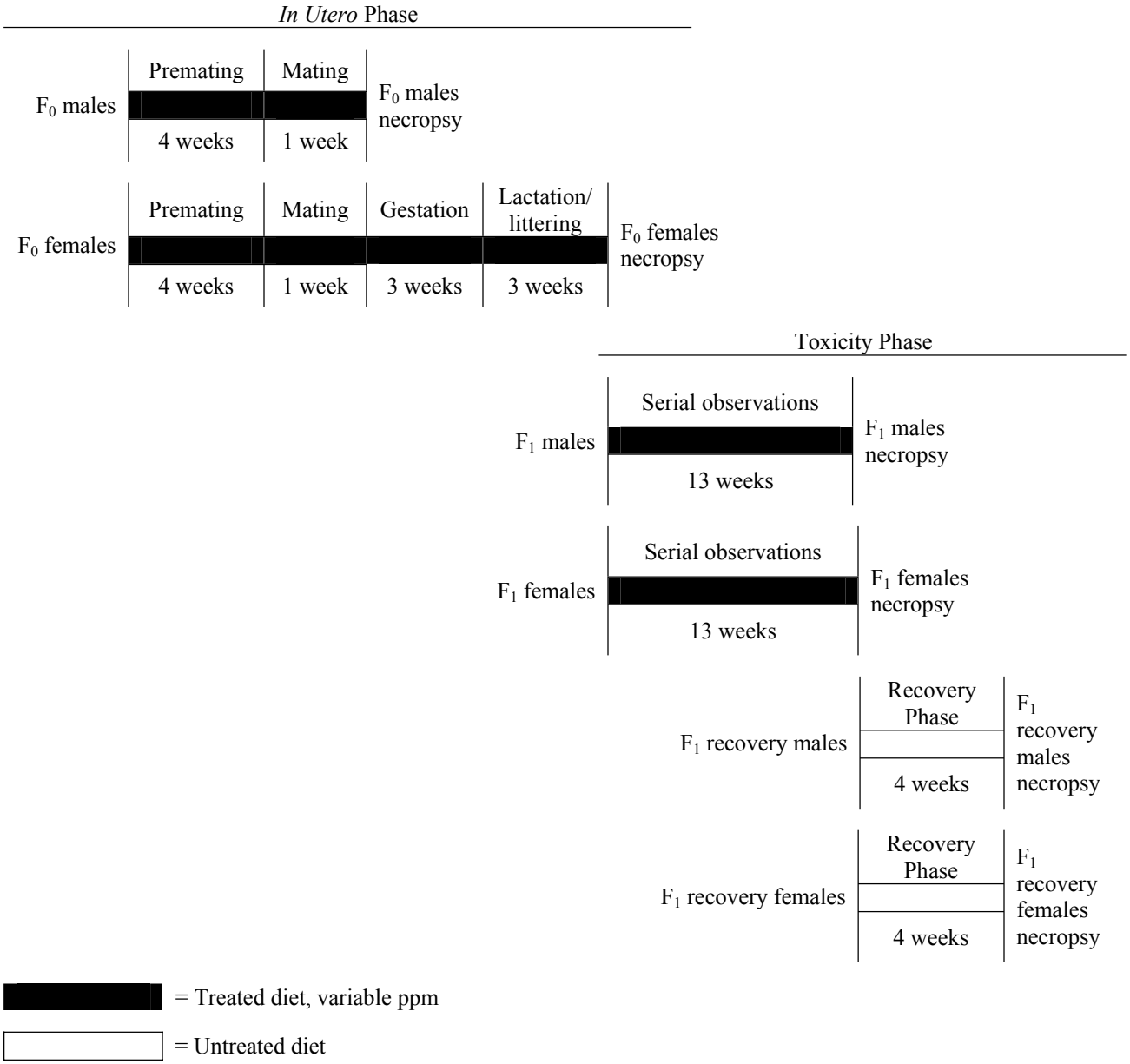
Method

Method/guideline followed:	US EPA OPPTS 870.3100; OECD Method 408; EC Method B26
Test type:	Oral
GLP:	Yes
Year:	2002
Species:	Rat
Strain:	Han Wistar
Route of administration:	Oral (feed)
Duration of test:	F_0 males and females: 4 weeks premating, mating; F_0 females: through gestation and lactation; F_1: 13-weeks plus a 4-week recovery period.
Doses/concentration levels:	0, 100, 300 and 1000 mg/kg/day
Sex:	Males and females
Exposure period:	F_0 males and females were treated for four weeks prior to mating until scheduled sacrifice. The F_1 male and female offspring were exposed *in utero* and from birth until the start of the 13-week study. The F_1 offspring selected for the 13-week study were then provided the respective treated diets for 13-weeks.
Frequency of treatment:	Continually, *ad libitum*
Control group and treatment:	Yes; untreated diet
Postexposure observation period:	4 weeks
Statistical methods:	For organ weights and body weight changes, homogeneity of variance was tested using Bartlett's test followed by Behrens-fisher test or Dunnett's test as appropriate. Macroscopic pathology and histopathology data were assessed using Fisher's Exact test. Estrus cycles were analyzed using the Cochran-Armitage trend test. Other statistical tests used as appropriate were: Williams' test for a dose-related response; Student's t-test; Shirley's non-parametric test for a dose-related response; Steel's test; and Wilcoxon rank sum test. Significance level was $p<0.05$.
Remarks:	The study design is shown in Figure 1. Parental (F_0) animals (25 rats/sex/group) were exposed to Citroflex® A-4 (ATBC) continuously in the diet at target doses of 0, 100, 300 and 1000 mg/kg/day for four weeks before

pairing and throughout mating. F_0 males were sacrificed after mating and treatment of the F_0 females continued throughout gestation, littering and lactation until they were killed after the litters were weaned on lactation day 21. The offspring (F_1 generation) were exposed to the test material *in utero* and from birth until the start of the 13-week study (when the animals were approximately four weeks of age). Details of the 13-week study are provided in the summary of this study for endpoint 5.4 Repeated Dose Toxicity.

Parental animals were evaluated for reproductive endpoints (mating performance, fertility, gestation length and parturition, litter size, numbers of implantations, survival and growth), and F_1 animals were evaluated for sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males, sperm assessments), estrous cyclicity, physical appearance, ophthalmologic effects, neurobehavioral effects, growth, food consumption, survival, hematology, blood chemistry, urinalysis, peroxisome proliferation, organ weights, gross pathology and histopathology.

Study Design for 13-Week Toxicity Study with an *In Utero* Exposure Phase



Results

NOAEL (NOEL)	NOAEL (parental) = 300 mg/kg/day NOAEL (offspring) = 1000 mg/kg/day
LOAEL (LOEL)	LOEL (parental) = 1000 mg/kg/day LOEL (offspring) > 1000 mg/kg/day
Actual dose received:	103, 306 and 1013 mg/kg/day for males 102, 306 and 1024 mg/kg/day for females
Toxic response/effects:	Described below
Statistical results:	Described below
Remarks:	The general condition of parental animals was unaffected by treatment. Females in the 1000 mg/kg/day dose group showed an increased incidence of yellow staining in the perigenital and sacral regions. Body weight, body weight gain, food consumption and food conversion efficiency were unaffected. Estrous cycles, mating performance, fertility, gestation length and parturition, were all unaffected by treatment. Litter size, survival and growth were similar in all groups and within expected historical control ranges. Although numbers of implantations and litter size at 1000 mg/kg/day were marginally lower than concurrent control group levels, they were within the laboratory's historical control ranges. Anogenital distance and sexual maturation in both sexes and retention of areolae in male offspring were unaffected by treatment. There were no adverse effects on sperm motility, counts or morphology. There were no findings at necropsy of parental animals or surplus offspring that were considered to be treatment-related. At the completion of the *in utero* phase, rats that had been exposed to ATBC from before conception, through gestation and continuously from the time of birth were selected (20 unrelated males and 20 unrelated females per dose group for the main study; and 10 unrelated males and 10 unrelated females for the control and high dose recovery groups) and transferred to the 13-week study. The results of the 13-week study phase are discussed in the summary of this study for endpoint 5.4 Repeated Dose Toxicity.

Conclusions

Remarks: The no-observed-adverse-effect levels (NOAELs) for reproduction and developmental toxicity for ATBC in this 13-week toxicity study with an *in utero* exposure phase were considered to be 300 mg/kg/day for parental animals and 1000 mg/kg/day for offspring, respectively. (Morflex, Inc.)
The endpoint has been adequately characterized. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 1A
Remarks: Reliable without restriction; modified guideline study

References

Chase, K. R. and C. R. Willoughby. 2002. Citroflex® A-4 Toxicity Study by Dietary Administration to Han Wistar Rats for 13 Weeks with an *In Utero* Exposure Phase Followed by a 4-Week Recovery Period. Project No. MOX 002/013180. Huntingdon Life Sciences Ltd., UK.

Other available reports

Other

Last changed: December 9, 2003
Order number for sorting:
Remarks:

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Species:	Rat
Strain:	Not stated
Route of administration:	Oral (diet)
Doses/concentration levels:	0, 250, and 500 mg/kg
Sex:	Male and Female
Exposure period:	Daily
Frequency of treatment:	*ad libitum*
Control group and treatment:	Yes, treatment not specified
Duration of test:	12 months
Statistical methods:	Not stated
Remarks:	Groups of animals were provided feed containing a milk solution of the test substance (ATBC) at doses of 50 and 250 mg/kg for 12 months. A third group served as a control. In the ninth month of the study, a cross-mating of the animals was performed, male gonads were evaluated and embryotoxic effects were examined. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (determined by examining the numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings; eye openings; appearance of body hair and teeth; behavior; and body weight.

Results

Parental toxicity NOEL:	50 mg/kg
Developmental toxicity NOEL:	250 mg/kg
Actual dose received:	Not stated
Parental data:	There were no effects of treatment noted in the animals provided the test substance in the feed corresponding to doses of 50 mg/kg. Toward the end

of the experiment, practically no single indicator evaluated in the ATBC-treated groups differed substantially from that of the control group animals.

ATBC had no significant effects on male gonads, and the spermatogenesis index in animals of the 250 mg/kg group was similar to controls.

Progeny data: Increases in body weight and length of the progeny and placental weight were observed in the 250 mg/kg dose group. There were no differences between groups in the fertility rate and number of animals born per pregnant female. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of the progeny also were unaffected by treatment.

Statistical results: Not stated

Remarks: This study showed that the chronic ATBC treatment of experimental animals resulted in no significant effects of the male sexual cells, had no embryotrophic effects and did not affect the growth and development of the progeny. (Authors of report)

Conclusions

Remarks: This study provides information on the repeated dose, reproductive and developmental toxicity of ATBC. (Morflex, Inc.)

ATBC is rapidly and extensively absorbed, and then rapidly metabolised and virtually completely excreted by the rat. Developmental toxicity was not observed at dose levels as high as 1000 mg/kg/day in a two-generation reproductive toxicity study nor in a 13-week toxicity study with an *in utero* exposure phase. The metabolites that have been positively identified in the urine of rats (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and two isomers of acetyl dibutyl citrate) have been demonstrated to undergo rapid clearance from the body and are not suspected to be developmental toxicants. Also, other ATBC metabolites, acetic acid, citric acid, butyric acid, tributyl citrate and butanol, do not pose a concern for developmental toxicity.

(See also, *5.8 Toxicity to Reproduction* and *5.10 Additional Studies*)

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; data are reliable but reference lacks details.

References

Larionov, A. G. and T. E. Cherkasova. 1977. Toxicological evaluation of Acetyltributylcitrate. Gig. Sanit., 4:102-103.

Other

Last changed:	December 9, 2003
Order number for sorting:	
Remarks:	

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
GLP:	Not stated
Year:	Not stated
Species:	Mouse
Strain:	Not stated
Route of administration:	Oral (diet)
Doses/concentration levels:	0, 250, and 500 mg/kg
Sex:	Male and Female
Exposure period:	Daily
Frequency of treatment:	*ad libitum*
Control group and treatment:	Yes, treatment not specified
Duration of test:	12 months
Statistical methods:	Not stated
Remarks:	Groups of animals were provided feed containing a milk solution of the test substance (ATBC) at doses of 50 and 250 mg/kg for 12 months. A third group served as a control. In the ninth month of the study, a cross-mating of the animals was performed, male gonads were evaluated and embryotoxic effects were examined. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (determined by examining the numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings; eye openings; appearance of body hair and teeth; behavior; and body weight.

Results

Parental toxicity NOEL:	50 mg/kg
Developmental toxicity NOEL:	250 mg/kg
Actual dose received:	Not stated
Parental data:	There were no effects of treatment noted in the animals provided the test substance in the feed corresponding to doses of 50 mg/kg. Toward the end

of the experiment, practically no single indicator evaluated in the ATBC-treated groups differed substantially from that of the control group animals.

ATBC had no significant effects on male gonads, and the spermatogenesis index in animals of the 250 mg/kg group was similar to controls.

Progeny data: Increases in body weight and length of the progeny and placental weight was observed in the 250 mg/kg dose group. There were no differences between groups in the fertility rate and number of animals born per pregnant female. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of the progeny also were unaffected by treatment.

Statistical results: Not stated

Remarks: This study showed that the chronic ATBC treatment of experimental animals resulted in no significant effects of the male sexual cells, had no embryotrophic effects and did not affect the growth and development of the progeny. (Authors of report)

Conclusions

Remarks: This study provides information on the repeated dose, reproductive and developmental toxicity of ATBC. (Morflex, Inc.)

ATBC is rapidly and extensively absorbed, and then rapidly metabolised and virtually completely excreted by the rat. Developmental toxicity was not observed at dose levels as high as 1000 mg/kg/day in a two-generation reproductive toxicity study nor in a 13-week toxicity study with an *in utero* exposure phase. The metabolites that have been positively identified in the urine of rats (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and two isomers of acetyl dibutyl citrate) have been demonstrated to undergo rapid clearance from the body and are not suspected to be developmental toxicants. Also, other ATBC metabolites, acetic acid, citric acid, butyric acid, tributyl citrate and butanol, do not pose a concern for developmental toxicity.

(See also, *5.8 Toxicity to Reproduction* and *5.10 Additional Studies*)

Data Quality

Reliability (Klimisch):	2D
Remarks:	Reliable with restrictions; data are reliable but reference lacks details.

References

Larionov, A. G. and T. E. Cherkasova. 1977. Toxicological evaluation of Acetyltributylcitrate. Gig. Sanit., 4:102-103.

Other

Last changed:	December 9, 2003
Order number for sorting:	
Remarks:	

5.10 ADDITIONAL STUDIES

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	99.02%
Remarks:	The ^{14}C-ATBC had a radiochemical purity of 100%

Method

Method/guideline followed:	Not stated
Type:	Absorption, metabolism and excretion
GLP:	Yes
Year:	1992
Species/Strain:	Sprague-Dawley rat
Sex:	Male
No. of animals per sex per dose:	4 (absorption and elimination study) 5 (rate of absorption study)
Vehicle:	Corn oil
Route of Administration:	Oral (gavage)
Doses/concentration levels:	70 mg/kg
Frequency of treatment:	Single dose
Duration:	48 hours
Remarks:	*Test substance preparation*: On the day prior to dosing, radiolabeled test article (^{14}C-ATBC) and unlabeled test article (ATBC) were dissolved in corn oil to provide a target dose of 70 mg/kg and 200 μCi/kg when 2 ml of the dosing solution was given per kg of body weight. The dose solutions were analyzed for ATBC concentration by HPLC and for radioactivity by liquid scintillation counting. *Absorption and Elimination Study*: Four male rats (211 to 255g) were given a single oral gavage dose of approximately 70 mg of ^{14}C-ATBC per kg body weight. Rats were fasted from food 16 hours prior to dosing until 4 hours after dosing. After dosing, the animals were housed in glass metabolism cages and urine, feces, and expired organics and $^{14}CO_2$ were collected at specified intervals (defined below) throughout the 48 hours postdosing. Urine voided during successive 12 hour intervals was collected in dry-ice chilled containers and analyzed by liquid scintillation counting for excreted ^{14}C-ATBC. After each urine collection the cage was rinsed with water to reduce carry-over between urine collections. The rinse water was also analyzed for ^{14}C-ATBC (results reported with urine totals). Aliquots of the 0-12 and

12-24 hour urine collected were pooled for each animal and analyzed by HPLC and GC/MS to identify metabolites and/or unchanged ^{14}C-ATBC. Feces were collected at 24 hour intervals in dry-ice chilled containers and analyzed for radioactivity. Aliquots of each 0-24 hour fecal homogenate were also pooled, extracted, and then analyzed as described for the urine. To determine elimination of ^{14}C via the lungs, the air exiting the metabolism cage was passed through a volatile organic trap and a $^{14}CO_2$ trap. The traps were changed at 6 hour intervals for the first 12 hours then 12 hour intervals for the remainder of the 48 hour post dosing period. The rats were sacrificed 48 hours after dosing and samples of blood, fat, GI tract (including contents), kidney, liver, skin and the remaining carcass were analyzed for radioactivity by liquid scintillation counting.

Rate of Absorption Study: Five male rats were fitted with an indwelling jugular vein cannula while under methoxyflurane anesthesia on the day prior to dosing. The rats (211 to 255g at the time of dosing) received a single oral dose of ^{14}C-ATBC as described above. After dosing, the rats were housed in glass metabolism cages. Blood was collected from the indwelling jugular vein cannula of each rat 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48 hours postdosing. All blood samples were analyzed for radioactivity and only those samples collected through 8 hours and at the end of the 48 hour period were analyzed for unchanged ATBC. Rats were fasted from food 16 hours prior to dosing and food was returned *ad libitum* 4 hours after dosing. The animals were sacrificed 48 hours post-dosing without further evaluation.

Results

Results: Measured levels of ATBC and radioactivity in the dosing solutions ranged from 90 to 115% of the target concentrations. No signs of toxicity were observed following dosing. One rat in the Rate of Absorption Study died following blood collection. The probable cause of death was complication from the blood collection procedure.

Absorption and Elimination Study: Between 99 and 102% of the administered radioactivity was recovered in the urine, feces, cage wash, expired CO_2, tissues, and carcass by study end (48 hours). The following table provides the results of the recovered radiolabeled material:

Route of excretion	% of Recovered ^{14}C
Urine (and cage rinse)	59 to 70%
Feces	25 to 36%
Expiration of $^{14}C0_2$	2%*
Tissues and carcass	0.36 to 1.26%

* No radioactivity was expired as radiolabeled volatiles other than $^{14}CO_2$.

Rate of Absorption Study: Absorption of the radioactive dose was rapid (absorption $t_{½}$ = 1.0 hour) and extensive (at least 67% of ^{14}C dose absorbed). Peak concentrations of radioactivity in blood were observed 2 to 4 hours post-dosing. Most of the absorbed radiolabel was rapidly eliminated with a half-life of 3.4 hours for blood. Metabolism of absorbed ^{14}C-ATBC was rapid and essentially complete. At least 9 radiolabeled metabolites were found in urine and at least 3 in feces. The labeled metabolites in urine were more polar than ATBC and less polar than citric acid. Urinary metabolites of ATBC which were positively identified were acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate, and acetyl dibutyl citrate (two isomers). The major labeled urinary metabolite was tentatively identified as monobutyl citrate. Unchanged ATBC representing about 7% of the dose was found in feces.

Conclusions

Remarks: Orally administered ATBC is extensively absorbed and rapidly metabolized and excreted by the rat (Author of report).

The study provides information on the absorption, metabolism, and excretion of ATBC in the rat. (Morflex, Inc.)

Data Quality

Reliability (Klimisch):	1B
Remarks:	Reliable without restriction; comparable to guideline study.

References

Dow Chemical Company. 1992. Metabolism and disposition of acetyl tributyl citrate in male Sprague-Dawley rats. Sanitized Laboratory Report. [Name of Testing Facility not stated].

Other available reports:

Other

Last changed:	December 3, 2003
Order number for sorting:	
Remarks:	

5.10 ADDITIONAL STUDIES

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	Tributyl citrate (TBC) and *n*-Butanol also were evaluated.

Method

Method/guideline followed:	Not stated
Type:	Metabolism
GLP:	Not stated
Year:	1991
Vehicle:	DMSO
Doses/concentration levels:	100 µg ATBC/ml (248 nmoles/ml) 100 µg TBC/ml (252 nmoles/ml) 14.8 µg n-butanol/ml (200 nmoles/ml)
Remarks:	The studies were performed to demonstrate that human serum and rat liver homogenates are capable of the metabolism of ATBC and its metabolite TBC and that butanol is a stoichiometric metabolite of both.

Human serum (50 ml) was secured from a single volunteer by venous puncture. The serum was stored at 4°C (never frozen) for three hours prior to use. Rat liver homogenate was obtained as follows: one 250 g adult rat was anesthetized with ether and the liver removed. The liver was thoroughly washed (with phosphate buffer) and then minced and homogenized in the same buffer. The homogenate was centrifuged and the supernatant obtained. Protein determination indicated a protein concentration of 65 mg/ml. This homogenate was diluted with an additional 20 ml buffer to yield a final protein concentration of approximately 32 mg/ml. The homogenate was held at 4°C (never frozen) for 24 hours prior to use.

Procedures for human serum: The appropriate volume of the ATBC stock solution was added to 15 ml of human serum to yield a concentration of 100 µg (248 nmoles) ATBC per ml. Likewise, the appropriate volume of TBC stock solution was added to 15 ml of human serum to yield a concentration of 100 µg (252 nmoles) TBC per ml. The appropriate volume of the butanol stock solution was added to 3 ml of serum to yield 14.8 µg (200 nmoles) per ml. All three tubes

were incubated at 37°C in a circulating waterbath and aliquots were taken for analysis at 0 min., 20 min., 1, 2, 4, 6, 11, 24, and 48 hours. The 1.0 ml samples collected for ATBC and TBC analyses were immediately inactivated by the addition of 1.0 ml 0.1N HCl. Samples were frozen until analyzed by GC. For the analysis of butanol, samples of 250 µl were taken from each of the three incubation tubes (ATBC, TBC and butanol) and placed in a 6 ml headspace vial with 25 µl of 1.0N HCl and 250 µl of an *n*-propanol internal standard solution was added and the vials seals by crimp closure. All samples were frozen pending analysis by headspace GC.

Procedures for rat liver homogenate: The appropriate volume of the ATBC stock solution was added to 10 ml of homogenate to yield a concentration of 100 µg (248 nmoles) ATBC per ml. Likewise, the appropriate volume of TBC stock solution was added to 10 ml of homogenate to yield a concentration of 100 µg (252 nmoles) TBC per ml. The appropriate volume of the butanol stock solution was added to 2 ml of homogenate to yield 14.8µg (200 nmoles) per ml. All three tubes were incubated at 37°C in a circulating waterbath and aliquots were taken for analysis at 0, 15, and 30 min., and 1, 2, 4, and 9 hours. The 1.0 ml samples collected for ATBC and TBC analyses were immediately inactivated by the addition of 1.0 ml 0.1N HCl. Samples were frozen until analyzed by GC. For the analysis of butanol, samples of 250 µl were taken from each of the three incubation tubes (ATBC, TBC and butanol) and placed in a 6 ml headspace vial with 25 µl of 1.0N HCl and 250 µl of an *n*-propanol internal standard solution was added and the vials sealed by crimp closure. All samples were frozen until analyzed by headspace GC. The levels of butanol were not measurable with this method; therefore, a method was developed for the extraction and direct capillary GC analysis of butanol produced in the liver homogenate as follows: Methods as described above were employed to prepare the ATBC and TBC samples, but the butanol samples were prepared to yield concentrations of 800 nmoles per ml. All three tubes were incubated at 37°C in a circulating water bath and 1.0 ml aliquots were taken for analysis at 0, 15 and 30 min., and 1, 2, and 3 hours and were

immediately inactivated by the addition of 0.1 ml 1N HCl. Samples were frozen prior to direct capillary GC analysis.

Results

Results:

Human serum results with ATBC and TBC: The metabolism of ATBC in human serum was a linear decline in the concentration of ATBC of the 48 hour period, after which only 25% of the starting material remained. An estimated half-life of 32 hours was obtained. In addition, only traces of TBC were detected from the deacetylation of ATBC to TBC. The metabolism of TBC in human serum showed an exponential decline in the levels of TBC with complete conversion observed in the 24 hour sample. An estimated half-life of 4 hours was obtained.

Rat liver homogenate results with ATBC and TBC: The metabolism of ATBC in liver homogenate was linear and rapid decline in the concentration of ATBC the first hour of the 9 hour period examined. From the slope of the linear decline, an estimated half-life of only 10 minutes can be obtained. Not even traces of TBC were detected from the deacetylation of ATBC to TBC was seen. The metabolism of TBC in rat liver homogenate showed a nearly instantaneous and complete metabolism of TBC in 15 minutes. The metabolism was so rapid that the T_0 data indicated only 35 µg/ml even though 100 µg/ml was added. A repeat incubation was conducted to try to capture an earlier time point in the conversion, but a comparable value (42 µg/ml) was again obtained. Thus, a half-life of seconds could only be estimated.

Results with butanol capillary GC analysis in human serum and rat liver homogenate: Butanol levels generated from ATBC were maximal at 1 to 2 hours, representing a level of 279 nmoles/ml at 2 hours. This represents a 37% (279 nmoles/750 nmoles) of the theoretical amount produced. Butanol levels generated from TBC, also maximal at 1 to 2 hours yielded 436 nmoles/ml, or 58% (436 nmoles/750 nmoles) of the theoretical amount. Therefore, with 3 moles of butanol theoretically produced from one mole of ATBC or TBC, the amounts observed were 1.11 mole equivalents from ATBC and 1.74 mole equivalents from TBC.

Conclusions

Remarks: Both ATBC and the intermediate metabolite TBC undergo rapid metabolism in both human serum and rat liver homogenates, which would be expected to yield the principal metabolites acetic acid, citric acid and butanol. The butanol would then be expected to further oxidize to butanoic acid and assimilated by ß-oxidation. Although a direct stoichiometry of butanol formed from ATBC and TBC was not observed, these results are partially explained based on the fact that butanol also is metabolized in the rat liver homogenate at a rate of 37 nmoles/ml/hr. It also may be suggested that an initial single or double debutylation may yield products which are less readily hydrolyzed in the system; products which would be, as fully ionizable carboxylic acids, readily excreted *in vivo*. (Author of report).

The study provides additional information on the metabolism of ATBC. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A

Remarks: Reliable with restrictions; acceptable, well-documented study report which meets basic scientific principles.

References

Davis, P. 1991. Technical Report on the Metabolism of Acetyltributylcitrate (ATBC) and Tributylcitrate (TBC) in Human Serum and Rat Liver Homogenates. Univ. Texas, Austin, TX, USA.

Other available reports:

Other

Last changed: December 3, 2003

Order number for sorting:

Remarks:

5.10 ADDITIONAL STUDIES

Test Substance

Identity:	Acetyl tributyl citrate (CAS RN 77-90-7)
Purity:	Not stated
Remarks:	

Method

Method/guideline followed:	Not stated
Type:	Metabolism
GLP:	Not stated
Year:	1991
Vehicle:	DMSO
Doses/concentration levels:	155 nmole/ml in human serum and 132 nmole/ml in rat liver homogenate
Remarks:	Experiments were performed to investigate the esterase activity in serum and liver homogenate on the hydrolysis of acetyl-tributylcitrate (ATBC) for prediction of its metabolism *in vivo*. Procedures utilizing capillary gas chromatography were developed, validated, and used to determine the formation of intermediate metabolic products (n-butanol and tributylcitrate – TBC) of acetyl-tributylcitrate (ATBC). Human blood was collected and the serum fraction was stored in a refrigerated at 4°C. The serum was used within 24 hours of collection. Rat liver was homogenized in three volumes of phosphate buffer. The homogenate was centrifuged and the supernatant was frozen at -20°C und used within 2 months. Dimethylsulphoxide was used as the solvent for ATBC. The ATBC solution was diluted with serum and liver homogenate to obtain an estimated concentration of 155 nmole/ml and 132 nmole/ml, respectively. Samples containing 150 nmole/ml of 1-butanol in serum and liver homogenate were also prepared to study the alcohol dehydrogenase activity in the incubation media. The samples were incubated at 37°C and duplicate 1 ml samples were collected at 3, 15, 30, 60 minutes and 2, 4, 6, 24, and 46 hours. Samples were acidified with 1 ml 0.1 M hydrochloric acid within one minute to inhibit the further hydrolysis of ATBC. One of the duplicate samples was analyzed for intact ATBC and the hydrolysis product, TBC, by capillary gas chromatography. The other duplicate

sample and the samples fortified with n-butanol were analyzed for the hydrolysis product n-butanol by head-space capillary gas chromatography.

Results

Results: In human serum, ATBC was hydrolyzed relatively slowly (half-life approximately 7 hours) into the equivalent of 2 moles of n-butanol. One butyl ester group of ATBC did not appear to undergo hydrolysis, most probably due to the lower affinity for the butyl group at the 2 position.

Hydrolysis in rat liver homogenate took place much faster (half-life < 30 minutes). Approximately 2.3 moles of n-butanol were recovered. As shown by a separate experiment, this amount is an underestimation of the true recovery value, the loss of the analyte being due to its consumption by liver enzymes, such as alcohol dehydrogenase.

Conclusions

Remarks: The results of this study confirm that the end products of ATBC hydrolysis in humans are unquestionably citric, acetic and butyric acid. (Author of report). The study provides additional information on the metabolism of ATBC. (Morflex, Inc.)

Data Quality

Reliability (Klimisch): 2A
Remarks: Reliable with restrictions; acceptable, well-documented study report which meets basic scientific principles.

References

Edlund, P. O. and J. Ostelius. 1991. *In Vitro* Hydrolysis of Acetyl-Tributylcitrate in Human Serum and Rat Liver Homogenate. Kabi Invent/ Procordia OraTech for Procordia Oratec, Inc.

Other available reports:

Other

Last changed: December 3, 2003
Order number for sorting:
Remarks: